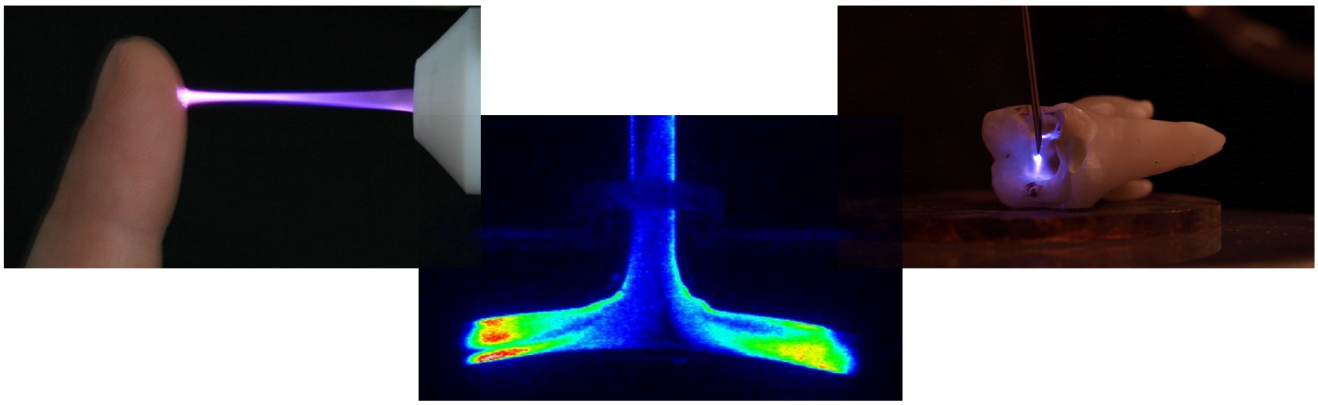
NATO Advanced Research Workshop

Plasma for bio-decontamination, medicine and food security



Jasná, Slovakia
March 15-18, 2011

Book of Abstracts

Edited by Karol Hensel and Zdenko Machala

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Cover pictures (left to right): Plasma jet in helium facing a human finger (author: I. Topala). 2 ns ICCD image showing the plasma separation and successive formation of two plasma "bullets" at a T junction (author: E. Robert). Human tooth cavity treated by nanosecond repetitively pulsed air discharge (authors: Z. Machala, D. Lacoste).

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Plasma for bio-decontamination, medicine and food security

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FOREWORD

Plasmas, especially non-thermal (cold) plasmas maintained close to room temperature, have recently found many breakthrough applications in biology, medicine, and security. Plasmas can efficiently kill bacteria, yeasts and molds and other hazardous microorganisms, including potential bio-terrorism agents, even spores and biofilms that are generally very difficult to inactivate. Cold plasma generated by electrical discharges can be employed for bio-decontamination and sterilization of surfaces, medical instruments, water, air, food, even of living tissues without causing their damage and other side effects, and represents a great potential in medicine and defense against terrorism. The sterilizing effect of plasma treatment can be attributed to several synergic mechanisms, including the UV radiation, electric field, charged particles, and generated radicals and reactive species. However, plasma induced biomedical processes are still mostly regarded as an efficient "black box." Deeper understanding of these mechanisms and their roles and synergies is absolutely necessary.

Direct or indirect plasma interaction with living cells of microorganisms or even humans is a new quickly developing field issuing in many bio-medical in vivo applications, e.g. for the treatment of foot ulcer and skin diseases. Cold plasma can also stop bleeding, making it effective in some surgical procedures and in treating intestinal ulcers and persistent nosebleeds. Enhanced blood coagulation and wound healing open up new vistas in military and defense applications. Plasma treatment also allows cell manipulations, their removal and targeted transfer into the injured area, which could also be used to accelerate wound healing. Plasma induced apoptosis (programmed cell death) of melanoma or other tumor cells in vivo and in vitro is being successfully tested, which brings forth a great potential for cancer treatment.

There is no doubt that multidisciplinary approach of plasma physicists, microbiologists, medical doctors and engineers is required here. The proposed advanced research workshop therefore aims at bringing experts in these fields together and letting them exchange their knowledge and interact. Such a gathering will potentially have an important impact on broadening the scientific and technological knowledge in this novel multidisciplinary area, on practical applications of plasmas in biology and medicine. It will expectedly promote close working relationships between scientists from different countries and with different professional experience.

We believe that beautiful Jasná mountain resort in Demänovská dolina, Slovakia, in late winter time will become a perfect place for a scientific, as well as a social aspect of this workshop. The workshop is sponsored by the NATO Science for Peace and Security programme and co-sponsored by the Faculty of Mathematics, Physics and Informatics of Comenius University in Bratislava. We are hosting 52 regular NATO-funded participants from 18 countries, thoroughly selected by the scientific committee based on their abstracts and following the NATO rules for advanced research workshops. Besides, several self-paid participants/observers and accompanying persons are participating. The scientific program comprises 10 key lectures, 27 oral presentations, and 21 posters. The event would not be possible without a hard work of 2 secretaries and 4 staff members of the local organizing committee.

This 144-page book contains 58 one- or two-page abstracts. No editorial corrections pertaining to the contents of the submitted abstracts were made and therefore the authors are fully responsible for the content of their contributions. The selected full papers, after successful peer review, will be published in a Springer book – NATO Science for Peace and Security Series – A: Chemistry and Biology.

Welcome to Jasná and deeply focus at hot scientific topics guarantied by the top class experts. Besides science, enjoy the surrounding mountains at 1200 m altitude, snow, food and drinks and especially the time spent together.

Zdenko Machala and Yuri Akishev,
NATO ARW co-directors

CONTENTS

KEY LECTURES

Prospects, problems and chances of the use of plasmas in life-sciences

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Résumé
An overview of atmospheric pressure plasma sources (APPS) capable for microbiological inactivation is given along with recent research results considering their antimicrobial effectivity.

Introduction

The inactivation of microorganisms and the removal of biological hazardous contaminants are generally of great interest in the entire field of life science. This varies from antimicrobial treatment of food or food containing enclosures, the conditioning of pharmaceuticals, the prevention or cure of infectious diseases, the bleaching of teeth, the installation of appropriate hygiene strategies up to the sterilization of spacecrafts. Nevertheless, the sterilization of medical instruments is still a main topic. In particular, the increasing application of complex and expensive medical devices like endoscopes or central venous catheters requires innovative sterilization methods that fulfill all demands for such high tech instruments. Generally speaking and not limited to the medical sector, an optimal sterilization process is safe, fast, cost-efficient, nontoxic and nonhazardous, environmentally friendly, energy efficient and does not stress the sterilized device or the containing materials. Although many sterilization methods are well established, adapted or even new methods are required due to the development of new techniques and instruments, the invention or enhancement of modern medical devices as well as the initiation of stricter hygienic standards in the field of life science. Low temperature plasmas generated at atmospheric pressure consist of a variety of microbiological active agents and are therefore appropriate tools for biological decontamination. As no costly vacuum equipment is required APPS are easily adaptable even to complex devices and conventional processes. Hence, their development and characterization is in the focus of research for more than two decades and until now the entire potential of APPS is unforeseeable yet.

Atmospheric pressure plasma sources for biological inactivation

Only plasma sources are presented, which are reported to be effective against biological contaminants. They are classified by their excitation frequency and electrode configuration: coronas, dielectric barrier discharges, atmospheric pressure plasma jets and microwave driven plasmas.

Corona discharges

Bussiahn et al. [1] presented an intermittent negative DC corona discharge denoted as hairline plasma. A pointed hollow needle electrode is feeded with argon gas and connected to a negative high voltage. Between the cathode and the anode, which usually consists of biological material, an intermittent plasma with a temperature of ~ 300K develops. The discharge produces ns-short current pulses with a repetition frequency of ~ 1.8 kHz and an amplitude of several hundred mA. The radial extension and the length of the plasma are 30 µm and up to 1.5 cm, respectively. The biological effectivity was demonstrated generally for the gram negative bacteria *E. coli.* Furthermore the ability of the hairline plasma to enter small cavities is impressively.

Dielectric barrier discharges (DBD)

Polak et al. [2] showed a special setup to generate a gas discharge inside a long and flexible tube for the use as biopsy channels in endoscopes by means of DBD. To provide an extended electric field along the tube, two electrodes are equidistant twisted around the tube with 2 mm inner diameter. The electrodes are located inside the tube wall, whereby the interior tube is not disturbed by foreign material and the outer side is electrically insulated towards peripheral devices. With this called bifilar helix discharge setup an uniform plasma could be ignited along a 5 m tube with inner diameter of 2 mm for various gas mixtures of

He, Ar, O_2, N_2. Preliminary results concerning the antimicrobial efficiency were achieved using a *B. atrophaeus* spores solution mixed with 0.3 % bovine serum albumin (BSA). It was demonstrated that a gas mixture of 1.5 slm argon and 200 sccm forming gas (95 % N_2, 5 % H_2) leads to a reduction factor of more than 4 $\log_{10}$ for an initial microorganism concentration of 10^6 CFU/ml and 10 min exposure time.

Hähnel et al. [3] used the remote impact of a surface DBD in ambient air for inactivation of microorganisms. Therefore, a special electrode geometry was invented. The antimicrobial efficiency was tested with *B. atrophaeus* spores at varying relative process gas humidities. The results show a strong dependence on the humidity. As for 30 % humidity a maximum of 1 $\log_{10}$ reduction could be reached, for 60 % relative air humidity all bacteria were killed. This is a reduction factor of 4 $\log_{10}$ for 10^5 CFU/ml initial concentration after 150 s plasma treatment. Additional pulse length variations indicate an exponential correlation between the plasma on-time and reduction rate.

Atmospheric pressure plasma jets (APPJ)

Ehlbeck et al. [4] and Weltmann et al. [5] show APPJ setup for inactivation of catheters. The generated discharge completely surrounds the outer surface of the catheter. The inactivation efficiency was tested by dividing the catheter into six sections and contaminating five sections with vegetative *Staphylococcus aureus* solution. The 6th section was kept as control. It reveals a 5 $\log_{10}$ reduction for pure argon and a complete inactivation of 6 $\log_{10}$ for argon with 0.25% air admixture. Additionally, the dependency of the amount of inactivation cycles was tested and no increase of efficiency could be detected.

Microwave driven discharges

Microorganism inactivation in PET-bottles is demonstrated by Brandenburg et al. [6]. They developed a self-propagating microwave-driven discharge at 2.45 GHz and power up to 1.7 kW in usual air. The device consists of a wave guide connected to the process chamber and an ignition device mounted on a moveable lance. The lance with the ignition pin is guided into the bottle, the microwave field is applied and a discharge is ignited at the bottom of the bottle. After the ignition the lance is moved to its origin and the discharge propagates upwards through the bottle. This was repeated three times, whereby one cycle takes about 550 ms, so 1.6 s for the entire process. The results show a 6.8 $\log_{10}$ reduction for *E. coli*, 5.1 $\log_{10}$ for *A. brasiliensis* and 6.7 $\log_{10}$ for *S. aureus*.

Conclusion

The special kind of the decontamination task is as manifold as the plasma sources. The varying plasma parameters and therefore the different composition of antimicrobial components in combination with complex microbial test methods using different microorganisms and procedures leads to an inhomogeneous picture. In consequence, each antimicrobial plasma treatment has to be carefully adapted to the specific task. This seems to be the reason for the limited utilization of plasma decontamination processes in industrial applications up to now. Currently, only few systems for very specific applications are commercially available. Another problem is that existing chemical disinfection and sterilization processes are cheap and effective. So plasma processes have to be even more time and cost efficient or have to occupy niches such as sterilization of endoscope channels. To overcome these difficulties more interdisciplinary research especially between physicist and biologist is needed. Due to the required high amount of reliability the involvement of engineers is desired.

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The prospects for atmospheric gas plasmas in the food industry

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Résumé

Despite the fact that recent years have seen an exponential growth in fundamental knowledge concerning the causative agents of food poisoning, incidences of this disease continue to grow in Western industrialized countries. In attempting to meet the changing demands of society for low-cost convenience foods it has become clear that the technology for ensuring its safe production has not kept up pace. In this presentation the various contributions that low temperature atmospheric gas plasmas can make to the production of safe food will be considered. Any process for decontaminating foodstuffs is subject to operational constraints; the nutritional value of the food must be preserved and its organoleptic properties must not be altered so as to render it unpalatable or unappealing to the consumer. In addition to examining the prospects which gas plasmas hold for microbially decontaminating foods themselves, the application of novel plasma configurations to food processing equipment – either to render it free of microbial contaminants or of residues that might cause allergic reactions in susceptible consumers – will be also be examined.

Damages of biological components in bacteria and bacteriophages exposed to atmospheric non-thermal plasma

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Résumé

Mechanism of inactivation of bio-particles exposed to non-thermal plasma, NTP, has been studied using *B. subtilis, E. coli* and bacteriophages. States of different biological components were monitored during the course of inactivation. Analysis of green fluorescent protein, GFP, introduced into *E.coli* cells proved that NTP causes a prominent protein damages without cutting peptide bonds. We have invented a biological assay which evaluates in vivo DNA damage of the bacteriophages. Different doses of the plasma were applied to wet state of λ phages. From the discharged λ phages, DNA was purified and subjected to in vitro DNA packaging reactions. The re-packaged phages consist of the DNA from discharged phages and brand-new coat proteins. Survival curves of the re-packaged phages showed extremely large *D* value (*D*=25 s) compared to the previous *D* value (*D*=3 s) from the discharged phages. The results indicate that DNA damage hardly contributed to the inactivation, and the damage in coat proteins is responsible for inactivation of the phages.

Introduction

There are strong concerns about the emergence of the next pandemic influenza, considering the recent worldwide outbreaks of highly pathogenic avian flu (H5N1) or swine flu (H1N1) and sporadic occurrence of human infections. Non-thermal atmospheric pressure plasma is hopeful about dealing with such menaces because, in principle, it can decontaminate both the surface of materials [1,2] and room air [3,4] at low gas temperature without using bactericides. Though the study of plasma decontamination is expanding [5-9], the intricacy and the high reactivity to any organic materials of the plasma sources have so far made it difficult to prove the inactivation mechanism of microorganisms.

In this study, we have invented a biological assay which evaluates DNA-specific damage in λ phages treated with cold plasma [10]. It was found that λ phage DNA was hardly damaged in the course of plasma application in comparison with easily affected coat proteins. We also evaluated the damages to *B. subtilis* and *E. coli* [11].

Strategy for assay of DNA damage

Generally, phage DNA by itself obtains total functions of the phage if it comes into the host cell. Therefore, in vivo function of the damaged phage DNA can be investigated by its transfection to the host cells. Figure 1 illustrates a concept for how to estimate the DNA-specific damage in plasma treated λ phages. Putative damage is introduced in both protein and DNA of the plasma treated phages (Phage A). DNA is extracted from Phage A and packaged in vitro to form newly packaged phages (Phage B). Phage B does not have any protein damage, but carries DNA damage originated from Phage A. Therefore, all of the damage for inactivation of Phage B belongs to DNA damage originally introduced to Phage A. Comparison of the *D* value (Decimal value: time for a one log10 reduction) of survival curves from Phage A and Phage B enables to evaluate the DNA damage, and as a consequence, to evaluate the protein damage.

Damage of the plasma treated λ phage having double-stranded DNA

λ phage suspended in 0.1M Tris-HCl (pH=8.0), 1×10^{-3}M of $MgSO_4$ was treated with the atmospheric pressure DBD. Inactivation profile (survival curves of Phage A) is shown as dotted line in Figure 2. The number of infectious phages decreased quickly and 6-orders of magnitude inactivation was achieved at 20 s discharge treatment. The profile exhibited the characteristic of a single slope curve and the *D* value was about 3 s.

The solid line in Figure 2 represents the survival curves obtained from the re-packaged λ phages (Phage B). Until 20 s discharge, the profile exhibited the characteristic of a single slope curve and the *D*

value was about 25 s. The large *D* value of re-packaged phages means very slow decrease of their viability. The shift of *D* value from 3 s to 25 s by re-packaging indicates that DNA damage introduced by the plasma hardly affected to the inactivation.

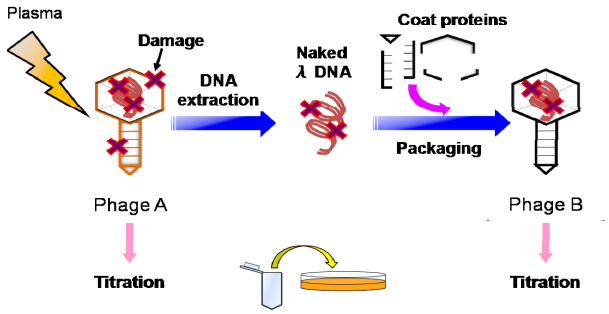

Fig. 1: Schematic procedure to estimate the DNA damage in plasma treated λ phages.

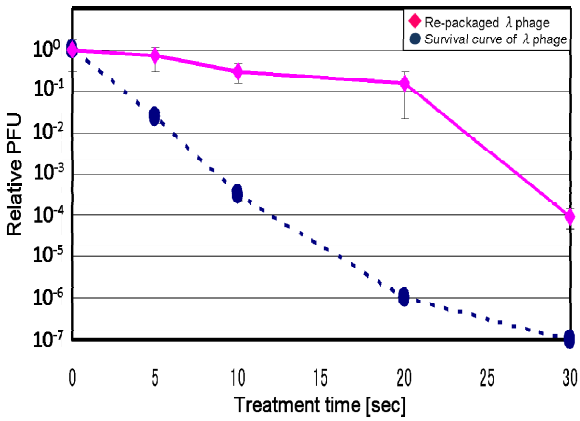

Fig. 2: Survival curves of λ phages subjected to the atmospheric pressure DBD. Dotted line represents the results from plasma-treated phage (Phage A). Solid line represents the results from re-packaged phage (PhageB). PFU: plaque forming units

Damage to the M13 phages having single-stranded DNA

For comparison, damages to M13 phages were evaluated. After the exposure to DBD, the DNAs were extracted, and were transfected to E.coli to measure the infection rate. The result indicated that the infection ability did not recover. The comparison shows that double-stranded DNA is stronger against the exposure to DBD, as the DNA repairment system of the host cell works if the damage remains on one strand.

Conclusion

λ and M13 phages, *E.coli* and *B. Subtilis spore* were inactivated effectively by the DBD exposure. Degradation of proteins seems major cause of inactivation for the microbes and λ phage. This damage could be denaturation or chemical modification such as oxidation or reduction but not degradation. The result showed that the double-stranded DNA of λ phage can be repaired. For M13 phage having single-stranded DNA, the repairmen system did not work, and degradation of both protein and DNA were observed.

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Plasma medicine

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Résumé

General principles of direct applications of non-thermal plasmas for medicine are discussed in the presentation, including physical, chemical and biological mechanisms of non-thermal plasma interaction with living tissues, plasma assisted sterilization, blood coagulation, and the non-thermal plasma-assisted healing of different diseases. The presentation summarizes the latest experimental results achieved by the plasma-medical group in the A.J. Drexel Plasma Institute. Both in-vitro and in-vivo plasma-medical experiments are discussed. From the entire variety of plasma-medical applications, this presentation is focused mostly on plasma-assisted treatment of wounds (both sterilization and healing).

Physics and engineering of all major types of cold discharges used in plasma medicine is to be discussed in the presentation. The major attention will be paid to the Floating-Electrode Dielectric Barrier Discharge (FE-DBD) and to the Pin-to-Hole Discharge (PHD). The FE-DBD is the main representative of the room- temperature atmospheric-pressure direct discharges applying treated tissue as the second active electrode. Physical, chemical and medical effects of the FE-DBD discharges are significantly dependent to the duration and shape of the applied voltage pulses, which influence plasma homogeneity and composition of the biologically active plasma-generated species. PHD represents the space non-homogeneous non-equilibrium discharges able to generate in addition to reactive oxygen species (ROS) the significant amount of nitrogen oxides (especially NO, and peroxynitrite). Gas-phase biologically active plasma-generated species are analyzed and discussed for both FE-DBD and PHD discharges.

Biological and medical effects of the non-thermal plasma on different kind of bacteria, mammalian cells, and on living tissue in general is very sensitive to the liquid-phase (including gels) interface separating the tissue from the gas-phase plasma. Experimental investigations of the biologically active components generated by plasma in the interface liquids are to be discussed. Biological responses of the tissue to the biologically active components generated by plasma in the interface liquids are to be analyzed in the presentation. Especial attention will be paid to the plasma-induced toxicity, DNA damage and related issues of the dose effect, safe treatment doses, and the selectivity of plasma treatment of the living tissue.

Discussion of the plasma medical approach to the wound healing will clarify specifics of different types of wounds, specifics of the plasma-induced wound sterilization versus the plasma-induced wound healing, effect of the plasma-tissue interface, toxicity of the plasma treatment, effects of high doses and plasma-induced burns, as well as effective depth of penetration of the plasma-treatment effects.

Antisepsis of the skin by treatment with tissue-tolerable plasma (TTP): Risk assessment and perspectives

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Résumé

The application of "cold" plasma is well suited for disinfection of non living materials. With "cold" plasma it is possible to decrease the microbial contamination of living tissues. In particular, when treating chronic wounds, it has several advantages in comparison to the classical application of antiseptics, which do not penetrate sufficiently into the tissue or inhibit wound regeneration. The mode of action of the plasma is based on the formation of free radicals, which destroy the bacteria and fungi. In the case of the investigated plasma-jet the present study could show that no electrical, UV or thermal damage of the skin can be expected, thus enabling an in vivo application.

Introduction

The application of cold atmospheric plasma is a promising method for wound healing [1-5]. It has antibacterial and antimicrobial effects and can stimulate fibroblast cells towards faster attachment and proliferation. In the present paper a risk assessment of cold plasma in dermatology is given.

In principle there are 3 properties of the plasma, which should be evaluated under the aspect of safety:

1. In the result of a gaseous discharge, radicals are produced on the skin surface. The human skin is continuously exposed to free radicals, which are produced by environmental factors like UV sun radiation. The human skin has developed a protection system against the destructive action of these highly reactive molecules in form of the antioxidative potential. The influence of the plasma on the antioxidative potential of the skin was investigated.

2. During the plasma formation, also UV radiation is produced. The spectrum of the plasma on the skin surface and in different depths of model tissue (pig ear skin) was investigated depending on different physical parameters influencing the formation of the plasma.

3. In the last series of experiments, the influence of the duration of the plasma treatment on the temperature of the skin surface was analyzed depending on different physical properties influencing the plasma parameters.

It was found that during the plasma treatment of tissue, the antioxidative potential is reduced only in the upper part of the stratum corneum, but not in deeper cell layers. Selecting the optimum parameters of the plasma formation, the UV exposure of the skin is less than in the case of UV irradiation of the sun on a summer day at noon.

If the duration of the plasma treatment of the skin is in the optimal range for wound healing, no thermal damage has to be expected.

The investigations have been carried out with an atmospheric pressure plasma jet (APPJ) working with Argon developed by INP Greifswald and neoplas GmbH.

Additionally the action mechanism of the plasma was investigated concerning the highly efficient in skin antisepsis [4-7]. The germs are not only located on the skin surface, but also in the hair follicles, from where they re-colonize the skin surface after antisepsis, e.g. It could be demonstrated that plasma is able to reach the follicular reservoir for antisepsis. For this purpose, a solution containing particulate chlorophyll dye had been applied onto porcine skin samples. The fluorescent properties of the dye changed during the plasma tissue interaction. The results demonstrate that TTP penetrates deep into the hair follicles, whereupon the hairs act as a conductor for the plasma. Therefore, it can be concluded that micro-organisms of the follicular reservoir are destroyed more efficiently by the plasma than by conventional liquid antiseptics.

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Biological effects of ultrashort pulsed electric fields

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Résumé

Exposures of cells to pulsed electric fields of ultrashort duration (nanoseconds) and high field strengths (tens of kilovolts per centimeter) can alter cell morphologies and functions. On this basis, applications can be devised for bacterial decontamination, wound healing, and cancer treatment.

Introduction

Exposures of cells in electric fields are known for the various responses, which depend on the specific parameters of the applied electric fields, in particular field strength and duration. Electromagnetic radiations with frequencies of hundreds of kilohertz (i.e. radio frequencies) up to some gigahertz (microwaves) are becoming main stream therapies to induce hyperthermia in tumors or to ablate tissues. Results are achieved by increasing the temperature at the target site to much higher levels than are tolerable for mammalian cells. Biological responses for power levels that do not lead to significant heating (or even a measureable increase in temperature) are discussed controversial, and are mostly associated with hazards from cell phone use and risks of ambient extremely low frequency electromagnetic fields (ELF).

Conversely, pulsed electric fields are used to deliberately induce non-thermal responses. Pulses with durations of microseconds to milliseconds are used to open cell membranes for the transfer of otherwise membrane impermeable large molecules, such as drugs or DNA [1]. Whereas the longer pulses more or less exclusively affect the outer cell membranes, nanosecond pulsed electric fields (nsPEFs), can in addition effectively reach organelles and subcellular structures [2,3]. The subsequent observed biological response depends on the specific cell (cell type and environment) and details of the applied stimulus (pulse duration, pulse amplitude, pulse number and pulse repetition rate). Consequently, different exposure schemes have been developed for various applications. The induction of subcellular effects can be used for the killing of bacteria or just the transient inactivation, i.e. stunning of microorganisms [4]. As trigger for biochemical cascades, nsPEFs can cause functional changes, and for example initiate the aggregation of platelets [5]. Another most promising response, is the induction of apoptosis, which holds the promise of new tumor therapies [6,7].

Interaction of nanosecond pulse electric field exposures with cells

The immediate responses of mammalian cells to the exposure in a pulsed electric field can be attributed to the charging of the plasma membrane. When the transmembrane voltage reaches a cell-line specific threshold value, which is generally on the order of 1 V, pores form in the membrane that allow ions and larger molecules like DNA to cross into and out of the cell. The mechanism is known as electroporation, refering to the application of pulses of several microseconds to milliseconds. If the duration of the pulse is shorter than the charging time of the outer membrane and pulse amplitudes are high, also membranes of internal organelles will be affected in addition to the plasma membrane. Depending on pulse parameters, the results of this stimulation vary, from a mere disturbance of cell function to the induction of apoptosis. To investigate the conditions for the induction of all these physiological reponses, we recorded the changes of the transmembrane potential during the application of a nanosecond pulsed electric field in real time, i.e., with a temporal resolution of 5 ns – short compared to the duration of the pulse. The results show that the intense fast-rising pulses lead to an immediate change of about 1 V at the anodic side of the cell. The subsequent development depends on the pulse amplitude. Once a value of 1.4-1.6 V is reached, decreasing transmembrane changes indicate the formation of pores, as was anticipated by modeling efforts. The observed changes in transmembrane potentials correspond to the increase of intracellular calcium levels several milliseconds after exposure for the same pulse conditions. Only for the highest electric field strength is this response dependent on the presence of extracellular calcium. This suggests that, except for this last case, calcium is released mostly from the cell's internal stores. The fast rise in

calcium in about 2-4 ms again suggests the outflow through pores. Ultimately, a better understanding of these ultrafast, non-physiological responses will allow us to manipulate cells to obtain a specific desired effect.

Environmental and medical applications of nanosecond pulsed electric field exposures

Pulses in the range of 0.1-100 kV/cm amplitude have been shown effective in biofouling prevention by either causing cell lysis or transient inactivation. The electrical energy that is required for lysis of bacteria can be reduced by decreasing the duration of the applied pulses. Lysis of *E.coli* was achieved with duration as short as 60 ns. More complex organism, such as brine shrimp (*artemia salina*) responded to pulsed electric fields with a temporary inactivation, i.e. were being stunned. For a more than 2 minute inactivation, the energy that had to be delivered by a 60-ns pulse corresponds to an energy density of 2 J/cm^3. Interestingly, stunning could be achieved with a ten times lower energy, for a 5-μs. pulse. This might indicate that a reversible disturbance of neurotransmitter signals between cells has significant contribution to the inactivation.

Currently the most prominent application of nanosecond pulsed electric fields is the possible cure for certain types of cancer. In vitro experiments have shown that exposures can lead to the expression of caspases that are known to lead to apoptosis. Applied to tumors in vivo, in addition the disruption of angiogenesis has been observed. Other mechanisms and their synergy seems also possible. The first survival study in mice demonstrated the total remission of B16 murine melanoma tumors and the long term survival of the animals after multiple treatments with 300-ns pulses. The success was recently repeated for HEP 1-6 murine liver cancers treated with 100-ns pulses.

Exposures of solid tumors so far require subcutaneous needle electrodes, which can possible be developed into laparoscopic procedures. Alternatively reducing the pulse duration into the sub-nanosecond range enables driving wideband radiation sources, which can deliver high electric fields deep into the body. In pursuit of this goal, we investigated the effect of 200-ps pulses and concurrently the radiation characteristics of prolate-spheroidal antennas, when excited by such a pulse [8].

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Nitric oxide plasma sources for bio-decontamination and plasma therapy

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Résumé

One of the main products generated in atmospheric plasma sources is nitric oxide. The nitric oxide molecule is known as anti-bacterial agent from one side and the molecule providing signaling and regulation biological functions from the other side. Some applications of nitric oxide plasma devices for sterilization and plasma therapy will be illustrated and discussed in the presentation.

Introduction

One of the most exciting medical discoveries of the 1980s in the study of human physiology was the realization that nitric oxide (NO) is a short-lived, endogenously produced gas that acts as a signalling molecule in the body. Signal transmission by a gas, produced by one cell, which penetrates membranes and regulates the function of other cells is an entirely new principle for signalling in the human organism. Today it is known that NO is also a universal anti-microbial factor and has important roles in the function of many tissues and organs, from the cardiovascular system to the brain.

Nitric oxide generation

Exogenous NO gas for bio-decontamination or therapeutic applications could be generated by several methods. The easiest way to obtain NO is chemical synthesis in the reaction of ammonia oxidation. However, the storage of chemically synthesized NO gas for further applications is a complicated problem because NO radicals are not stable and could be converted to NO2 gas in the reaction of recombination in the presence of oxygen. That is why plasma chemical synthesis in the flow of atmospheric air could be an alternative method of NO generation for anti-bacterial and medical applications *in situ*.

Plasma device “Plason” based on arc discharge was developed for generation gas flow containing NO with different configurations of the exit channels corresponding to the different medical applications: blood coagulation, tissue destruction, therapeutic manipulator/stimulator. Plasma temperature and nitric oxide content at the anode exit differs in different configurations of the device, corresponding to different medical applications.

Anti-bacterial effect and nitric oxide plasma therapy

The great need of new anti-bacterial platform is determined by continues increase of multi-drug resistant bacteria. The new antibiotics are often far more expensive and more toxic than predecessors. In vitro investigation of the influence of NO on *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, and Candida albicans,* which are typically associated with many hospital infections, showed that treatment by NO practically removes them all. NO could be used also to kill antibiotic-resistant, fungal (*Tinea Pedis*) and parasitic infections (*Leishmaniasis*).

Effectiveness of the plasma NO-therapy is already at present shown with a number of diseases in wound pathologies (trophic ulcers, diabetic foot ulcer), gynecology, traumatology, stomatology, ophthalomology, otorhinolaryngology, dermatology, gastroenterology, etc. Some new specific medical applications of the NO plasma systems will be described in the presentation.

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First achievements and opportunities for cancer treatment approach using non thermal plasma

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Résumé

This abstract summarizes the experimental results obtained and plasma delivery strategy developed in GREMI for the evaluation of antitumor action of dielectric barrier discharge and plasma gun for cancer treatment. Detailed analysis of biological effects following cold plasma application for both in vitro and in vivo experiments reveals the role of ROS, DNA damage, cell cycle modification and apoptosis induction. Recent characterization of plasma splitting and mixing in capillary geometry, using the plasma gun, together with preliminary tolerance study dealing with lung and colon treatment indicate that endoscopic plasma delivery may be a new and valuable therapy in cancerology.

This work deals with the development of two non thermal plasma sources and their application in cancerology. A floating electrode dielectric barrier discharge device (FE-DBD) and a plasma jet generator, labelled plasma gun [1], are used for both in vitro and in vivo assessment of cold atmospheric pressure plasma as a new therapy for cancer treatment. The first experiments were performed using the FE-DBD as an external plasma applicator on mice subcutaneously grafted with a tumor. The operation of the FE-DBD at low electrical power, a few watt, and correspondingly moderate plasma flux on the mouse skin surface allow repetitive plasma delivering for a few minutes during a few consecutive days. Following this plasma application protocol neither systemic behaviour, cardiac and pulmonary rhythm alterations nor severe skin burns were measured during tolerance studies. A five day DBD plasma treatment was shown to induce a significant delay and slowing down of the growth of U87 glioma cancer, in comparison with non treated control group [2]. This first demonstration of in vivo plasma therapeutic effect on a resistant cancer target was at the origin of three main experimental investigations: the study of the plasma delivery protocol, in vitro studies on different cell lines and the development of a new plasma source likely to allow endoscopic treatments. In vitro studies concern U87 (brain tumor) and HCT116 (colon tumor) cell lines for which both FE-DBD and plasma gun were used. The two plasma sources were shown to be efficient to kill the cell after plasma application over periods of a few tens of seconds. A more detailed analysis of FE-DBD action mechanism in in vitro cancerous cell treatment has been performed. The crucial role of ROS was proven by the comparable effect of a direct DBD plasma exposure and an indirect treatment protocol for which the culture medium was exposed to plasma and then transferred in culture wells containing cancerous cells. The use of ROS scavenger confirms that these species are, in vitro, the main agent in the cell destruction. DNA damage characterization, apoptosis quantification, and cell cycle analysis have then been performed. Briefly, formation of DNA double strand break damages and an accumulation of cells in the S phase were measured. Significant enhancement of the portion of cells in an apoptotic stage was also measured following in vitro plasma treatment. Dealing with the in vitro effect of plasma, the analogy of our results with plasma exposure with conventional cancer treatment strategies such as radio and chemo therapies, leads to suggest the following scenario to explain the cancerous cell destruction under plasma action. The key element is associated with in situ ROS generation leading successively to DNA damage, cell cycle arrest, and apoptosis induction. A very promising but questioning results concern the effect of plasma treatment during in vivo application. Apoptosis increasing and cell cycle alteration leading to an accumulation of cells in the S phase, were also measured by analyzing in vivo plasma treated tumors with the same detection techniques. The figure 1 presents the cell cycle measurements for in vitro and in vivo studies. Comparable G0/G1 fraction decrease, S phase accumulation and G2/M level are detected for in vivo and in vitro treated cells in comparison with the control group distribution.

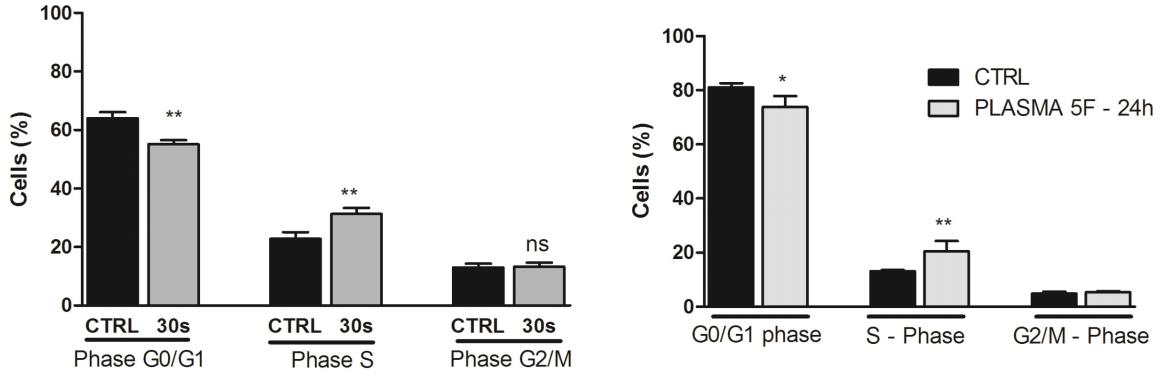

Fig. 1: Cell cycle distribution in control and plasma treated groups for in vitro (left) and in vivo (right) U87 tumor.

The role of ROS, reported to be of greater influence for in vitro plasma action, during in vivo treatment remains unclear, the main issue lying in the potential diffusion of these active species through the mouse skin to the tumor volume. In vivo plasma effects may also be correlated with plasma triggered in situ, i.e. in tumor environment, ROS release through local surface modification of charge density, temperature, or pH value. Such local pH modification was for instance observed on the skin surface where acidification occurs while subcutaneously a slight increase of the pH value was measured. Such chemical modification may induce in situ ROS release which may then interact with the tumor cells eventually through similar pathways as those encountered during in vitro trials. If this assumption sounds, the tumor treatment will probably be greatly optimized by delivering chemically active plasma in the close vicinity of the targeted cells. To check the efficiency of this mode of plasma application, there exists a need for a plasma source likely to allow endoscopic treatment. The main targets of our study concern colorectal and lung cancers for which access requires a specific care. The plasma gun set up is based on a pulsed nanosecond DBD reactor coupled with high aspect ratio flexible capillaries through which ionization wave sustained plasma propagation occurs. The possibility to use the plasma gun in multi branched volume, including branch splitting and connection at different locations, was recently experienced with success. Thus the plasma propagation was shown to be possible at large distances from the primary DBD plasma up to a few tens of centimetres through capillary structure exhibiting both splitting and connection of different branches. This unique specificity of the plasma gun among all other various plasma jet sources appears especially relevant for in vivo plasma application. The possibility to perform plasma delivery through micrometric catheter suitable for mice treatment by coloscopy or tracheotomy and flushed with low rare gas flow was recently achieved together with preliminary evidence for a reasonable tolerance of both colorectal and lung tissues under plasma exposure of a few minutes. The feasibility of such plasma application in combination with a proper matching of the plasma gun characteristics allows in situ in vivo study and optimization of antitumor action of cold plasma. Recent studies on the plasma jet action [3] on colorectal cell lines confirms cell growth arrest [4] and report a selective apoptosis induction for different tumoral and normal cells [5].

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Excilamps and atmospheric pressure plasma and their applications in biology and medicine

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Résumé

In the present paper the review of the results obtained at the Laboratory of Optical Radiation at High Current Electronics Institute SB RAS during 2008-2010 years is presented. Main attention pays to applications the excilamps and atmospheric plasma in biology and medicine.

Excilamps [1]

Efficient radiation of Ar_2, Kr_2, Xe_2, KrBr*, KrCl*, XeI*, XeBr*, XeCl*, Cl_2* molecules and I atoms was obtained in rare gas or in rare gas-Br_2(Cl_2, I_2) mixtures. Study of radiation parameters and lifetime period of the manufactured barrier discharge excilamp has been performed. Radiant power for the just sealed off KrCl ($\lambda \sim 222$ nm) excilamp exceeded 100 W was obtained. Maximal radiation power density was of 50 mW/cm^2. UV output power of ~75 W and efficiency up to 10 %, respectively, at $\lambda \sim 308$ nm (XeCl* excilamp) were obtained under excitation by pulses with frequency of 100 kHz. Application of water cooling allows increasing the radiant power of DBD coaxial excilamps. VUV radiant power of ~ 120 W at $\lambda \sim 172$ nm was obtained under excitation by pulses with frequency of ~ 100 kHz. The lifetime of gas mixtures in small-size XeCl and KrCl barrier discharge excilamps over 12000 and 8000 h was demonstrated. Dynamics of discharge formation in KrCl excilamp was studied. The extraordinary characteristics of excilamps led to a lot of applications, which had been demonstrated in a number of recent studies.

Atmospheric pressure plasma [2]

Breakdown of the gaps with a non-uniform electric field filled with air and nitrogen as well as with other gases under high-voltage nanosecond pulses was investigated. It is shown that conditions of obtaining a diffuse discharge without a source of additional ionization are extended at the voltage pulse duration decreasing. A volume discharge is formed due to the gap pre-ionization by runaway electrons and X-ray quanta. A runaway electrons preionized diffuse discharge (REP DD) has two characteristic stages. In the first stage, the ionization wave overlaps the gap during a fraction of a second. The second stage of the discharge can be related to the anomalous glow discharge with a high specific input power. At a negative polarity of the electrode with a small radius of curvature, a volume (diffuse) discharge formation is determined by pre-ionization with runaway electrons which are generated due to the electric field amplification near the cathode and in the gap. At a positive polarity of the electrode with a small radius of curvature, the X-ray radiation, generated at the runaway electrons braking at the anode and in the gap, is of great importance in a volume discharge formation. At the REP DD, the anode is influenced by the plasma of a dense nanosecond discharge with the specific input power up to hundreds of megawatt per a cubic centimeter, by the electrons beam, shock wave and optical radiation from discharge plasma of various spectral ranges, including UV and VUV. A REP DD is easily realized in various gases and at different pressures. This allows forecasting the REP DD application for disinfection, for producing ozone, for modification and cleaning of dielectric and metal surfaces.

Applications [3, 4]

UV inactivation of biological systems by excilamps. VUV or UV excilamps appear as an interesting option to conventional light sources for UV disinfection. Thus, one should distinguish between two different disinfection methods: the inactivation of microorganisms by UV irradiation (e.g. by KrCl*, XeBr*, KrBr* excilamps) or their total VUV-induced photomineralization (by Xe_2* excilamp). The comparative analysis of inactivation by excilamps and other means (plasma processing, laser irradiation, LP Hg lamps) has demonstrated that excilamps are the competitive technical systems. Excilamps based on XeBr* (283 nm), XeCl* (308 nm), KrCl* (222 nm), and Xe_2* (172 nm) molecules were used for

bactericide purposes. Recently, the inactivation effect of excilamps was demonstrated for a number of microbiological objects. In [4] it has been carried out a comparison of the bactericide properties of CD- and DBD-driven XeCl*, XeBr*, and KrCl* excilamps and has shown that a XeBr* excilamp (electric input power P_{el} of 60 W, UV radiant exitance 10 mW cm^{-2}) is the most efficient light source for inactivation of bacteria in this series. Concluding this chapter, let us note that some microorganisms and cells possess UVA/VIS repair mechanisms (photoreactivation) that substitute or dissociate thymine dimers. Under these circumstances, excilamps as narrow-band emission sources should be more efficient than wide-band MP Hg lamps. Sure, this problem needs further studies to be done.

UV phototherapy of skin diseases. One of the most effective methods of psoriasis curing is UVB phototherapy. Radiation is absorbed by endogenous chromophores, especially by DNA nucleotides, which lead to suppression of DNA synthesis in epidermal cells, for example in psoriatic plaques. Photochemical reactions of these molecules result in alterations of skin and then lead to the curing effect. Apparently, the DNA damage is the general mechanism at UV curing of skin diseases. Particularly, UV radiation affects the production of soluble mediators, the expression of cell-surface receptors, to induce apoptosis in pathogenetic relevant cells. More than 90% of the DBD XeCl* excilamp radiant energy is within the anti-psoriasis action spectrum. Thus, this excilamp is also a good variant for psoriasis curing, which was proposed for the first time in 1994 by Oppenlaender [3]. An example of UVB curing of psoriasis by the XeCl* excilamp is presented in Figure 1.

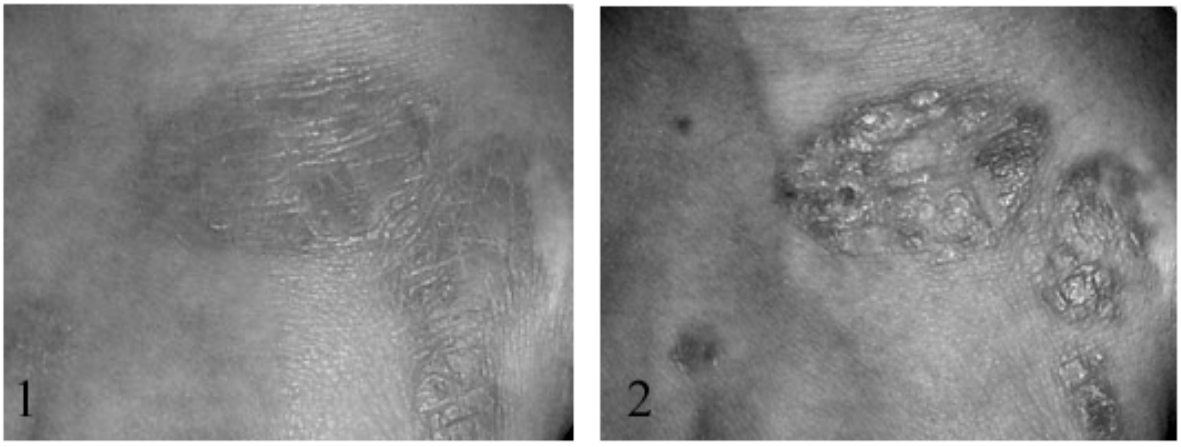

Fig. 1: Example of psoriasis curing by the XeCl* excilamp in Siberian Medical University (BD_compact model, Optical Radiation Laboratory, Russia, output window square 30 cm^2, UV photon exitance of 40 mW·cm^{-2}): after 10 days at suberythermogenic doses treatment (1) and before curing (2).

The merits of such a therapy method are a good tolerance by patients and the use of suberythermogenic doses. In comparison with a XeCl* laser, the excilamp is cheaper and simpler in use. There are no principal restrictions for XeCl* excilamp radiant area increase that allows to create major set-ups both for local and total-body irradiation.

*Narrow-band UVB/UVC photoregulation of plants***.** The conditions of plants cultivation is appreciably influenced by plant biochemistry. The light factor (radiant exitance, spectra) has a very strong regulatory impact on plants. The new facts about a regulatory action of UVB and UVC on coniferous plants, particularly, on accumulation of photosynthetic pigments in needles of Siberian cedar seedlings were obtained. It has been shown that narrow-band UVB from XeCl-excilamp is very interesting for study of increasing the productivity of plants.

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Features of the sterilization by UV irradiation of low-pressure discharge plasma

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Résumé

The report is devoted to peculiarities of sterilization of items by UV radiation of the discharge plasma both in case of the items immersed into the discharge plasma (direct treatment), and in case of flowing afterglow plasma (remote treatment).

Report content

1. Microbiological methodology:
   - Types of survival curves. Vitalistics and mechanistics conceptions.
   - Difference of the plasma sterilization from classic (moist and dry heat) ones, influence of surface density of spores/microorganisms on the plasma sterilization process.
2. UV sterilization of the items immersed into the plasma:
   - The most efficient UV radiation, influence of the gas content on spectrum of UV radiation of the plasma.
   - Influence of UV radiation spectrum shape on the sterilization efficiency. Weighing curves.
3. Sterilization by UV radiation of flowing afterglow of the plasma:
   - N_2-O_2 plasma;
   - Ar, O_2 plasmas, influence of the gas impurities on UV sterilization efficiency.
4. Synergy effect of heat and UV photons.

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ORAL PRESENTATIONS

Sterilization using pulsed corona microplasma jet

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Résumé

Atmospheric pressure pulsed corona microplasma jet has been used for the sterilization of endospores and E-coli.

Introduction

Microplasma jet for the generation of pulsed corona discharge at atmospheric pressure has been devised for sterilization as well as to modification surface properties. Long filament of plasma is generated inside a quartz tube. Its efficiency of sterilization on inner surface of the tube as well as on objects placed in front of jet was studied. Endospores and E-coli are used for the sterilization studies. Sterilization of endospores, which are placed in front of jet shows very good sterilization results. Sterilization of E-coli coated on inner surface of the tube shows the bacterial survival ratio is 0.0038 %. In addition to this, inhibition studies of bacteria coated on agar plate also carried out (figure 1 and 2). The results obtained in these studies will be presented along with possible sterilization mechanism derived from the plasma parameters.

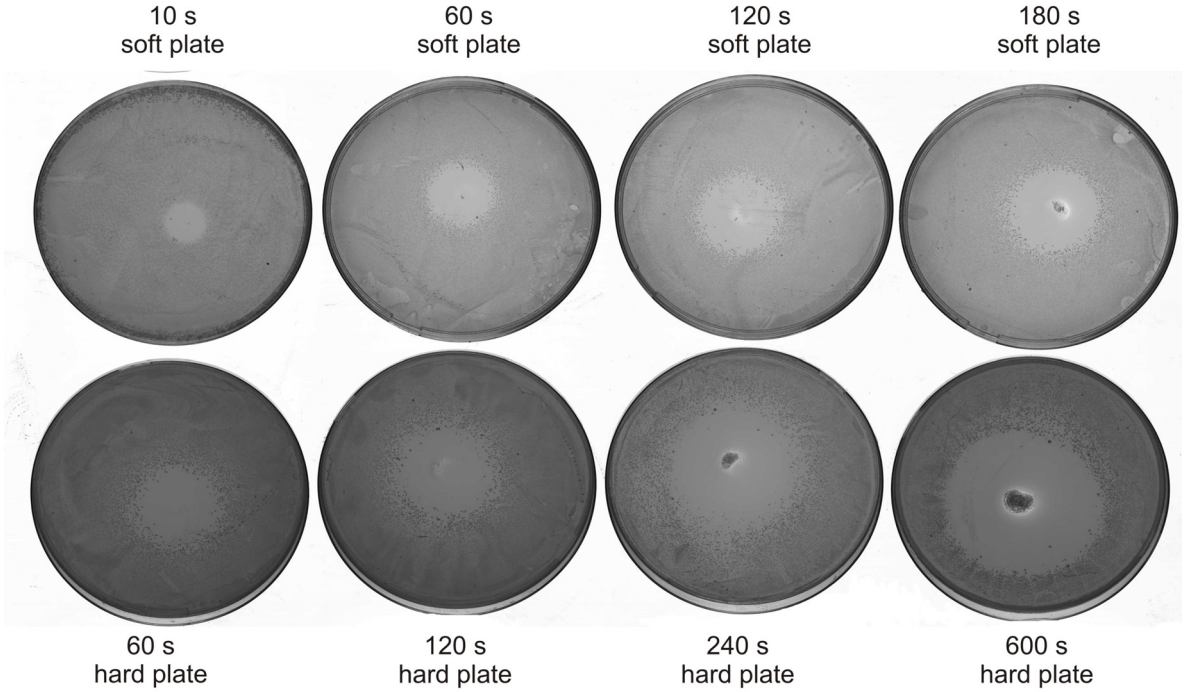

Fig. 1: Images of inhibition zone generated after plasma treatment of E-coli coated agar plates.

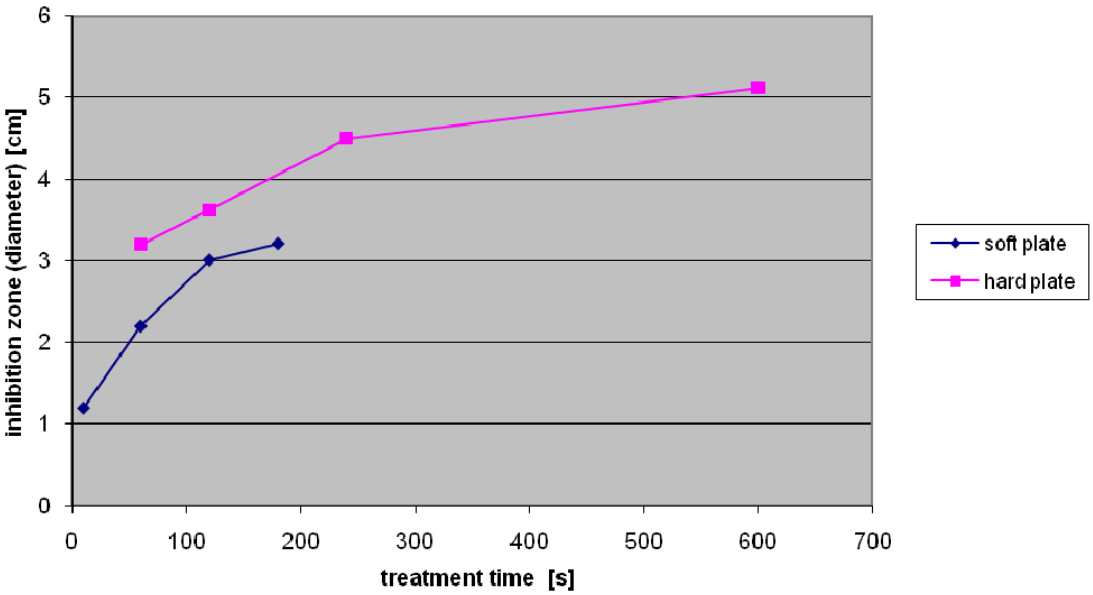

Fig. 2: Plot of inhibition area with respect to plasma treatment time on hard and soft agar plates.

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Biocidal effects of nanosecond repetitively pulsed discharges

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Résumé

Nanosecond Repetitively Pulsed (NRP) discharges are increasingly used in the cold plasma community for various applications such as plasma-assisted combustion or nanomaterials synthesis [1-3]. These discharges are particularly interesting because they can efficiently produce high concentrations of chemically active species such as atomic oxygen, nitrogen metastables, or ozone, in atmospheric pressure and ambient temperature air. In particular, we have shown recently that an NRP discharge can dissociate up to 50% of molecular oxygen with very high energy efficiency [4,5]. Given the importance of reactive oxygen species in biodecontamination, we investigate in the present work the effects of NRP discharges on bacteria inactivation in water, on teeth surfaces, and on agar plates. The NRP discharges are produced using short-duration (10 ns) high-voltage (4-10 kV) positive-polarity pulses at a pulse repetition frequency of 1-30 kHz across a discharge gap of a few mm. The electrode system is comprised of a needle (HV positive pulse) and a grounded plate or grid, in the configuration previously proposed by Machala et al. [6-7]. Two regimes of NRP discharges have been investigated: glow and spark [8]. Preliminary results indicate that both regimes produce a noticeable effect on the decontamination of the media studied. Although the spark regime is more effective, the glow regime has nevertheless a significant effect on bacteria despite the much lower power deposited.

Acknowledgments:

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Plasma-liquid-interactions: chemistry and antimicrobial effects

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Résumé

Plasma-induced inactivation of bacteria in aqueous liquids is supported by acidic pH and accompanied by generation of detectable chemical species like nitrate, nitrite and hydrogen peroxide. To get more insight into mechanisms of change of liquid composition by plasma treatment as well as transmission of bactericidal plasma effects into aqueous liquids, plasma diagnostics and liquid analytics are combined with theoretical considerations to focus possible reaction channels of plasma-water interactions.

Introduction

At the present state of knowledge, inactivation of bacteria in aqueous liquids by atmospheric pressure plasma treatment is strongly dependent on acidification (Fig. 1). However, it was also demonstrated recently that acidification alone does not induce comparable bactericidal efficacy. Moreover, plasma treatment of aqueous liquids results in additional changes of liquid composition, e.g. by generation of nitrate (NO_3^-), nitrite (NO_2^-) and hydrogen peroxide (H_2O_2) [1-4]. Therefore, the aim of further work is to get more insight into possible mechanisms of both liquid chemistry and bactericidal efficacy.

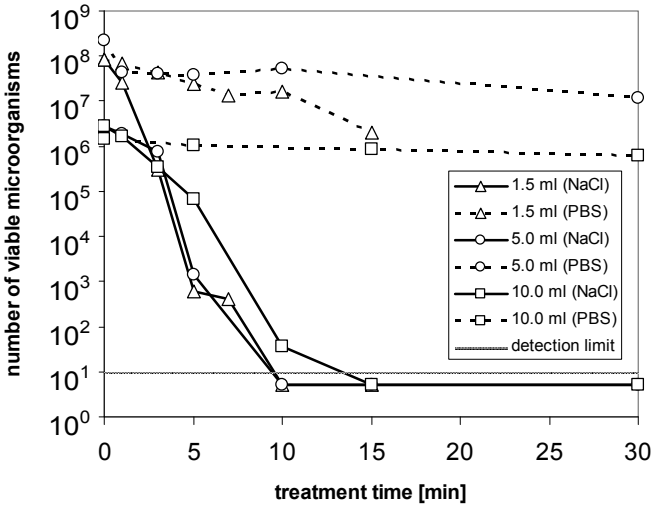

Fig. 1: Kinetics of inactivation of 1.5 ml (△), 5.0 ml (○) and 10.0 ml (□) *S. aureus* suspensions in physiological saline (NaCl; —) and phosphate-buffered saline (PBS; ---), respectively, by surface-DBD plasma (mean of n=2 each)

Experimental

Plasma treatment of aqueous liquids (distilled water, phosphate buffered saline, or physiological saline, respectively) was realized using a surface dielectric barrier discharge (surface-DBD) arrangement which was described in detail elsewhere [4]. Plasma is generated on the surface of an electrode array which was mounted by a special construction into the upper shell of a petri dish (60 mm diameter) in that way that a constant distance of 5 mm between the high-voltage electrode surface and the surface of a liquid sample in the lower shell of the petri dish (surface area 23.8 cm^2) can be adjusted. All experiments are performed at ambient air conditions using a pulsed sinusoidal voltage of 10 kV_{peak} (20 kHz) with a 0.413/1.223 s plasma-on/plasma-off time. Energy of 2.4 mJ was dissipated into the plasma in each cycle of high voltage. The power is 0.25 $W \cdot cm^{-2}$. Plasma diagnostics was realized using OES and FT-IR. For liquid analytics, UV/VIS spectrophotometer and semi-micro pH-electrode have been used. Detection of nitrite, nitrate and hydrogen peroxide in plasma treated water has been realized both by color forming reactions using chemical indicator substances and direct photometric analysis via total absorption spectra.

Results and discussion

Biological as well as chemical plasma effects in liquids must be a result of complex interactions at the plasma/gas-liquid interface and subsequent reactions in the liquid volume. To clarify possible mechanisms of chemical species generation as well as of microorganism inactivation in plasma-treated liquids, gas phase above the liquid surface was analyzed. Usually, direct or indirect actions of plasma-generated nitric oxide ($NO^{\bullet}$) or hydroxyl radicals ($HO^{\bullet}$) are considered to be mainly responsible for biological plasma effects. However, no $NO^{\bullet}$ was detected in the plasma/gas phase neither by OES via emission in the bactericidal effective UV-C range around 254 nm nor by FT-IR. Surprisingly there was also no emission of hydroxyl radicals ($HO^{\bullet}$) at 309 nm. This is in contrary to other studies [5] and could be explained by the low energy input realized with our discharge arrangement. Using FT-IR, stable molecules like nitrous oxide (N_2O), ozone (O_3), carbon dioxide (CO_2), and traces of nitric acid (HNO_3) and/or peroxynitrous acid (ONOOH) were measured. Reactions of these molecules from the plasma/gas phase with the aqueous liquid could result in acidification and generation of NO_3^-, NO_2^- and H_2O_2 via reactions which are associated with the occurrence of several more or less stable but biologically active chemical intermediates like $NO^{\bullet}$ or nitrogen dioxide ($NO_2^{\bullet}$). On the other hand, NO_3^-, NO_2^- and H_2O_2 could serve as starting reaction partners to generate $NO^{\bullet}$, $HO^{\bullet}$, $NO_2^{\bullet}$, ONOOH and hydroxyl radicals ($HO^{\bullet}$) in the liquid (Fig. 2). Consequently, by treatment of aqueous liquids by atmospheric pressure plasma at ambient air conditions a very complex chemical reaction cascade is induced resulting in a multi-component biologically active liquid "cocktail" containing $NO^{\bullet}$ and $HO^{\bullet}$ even if these reactive species are not detectable directly within the plasma/gas phase.

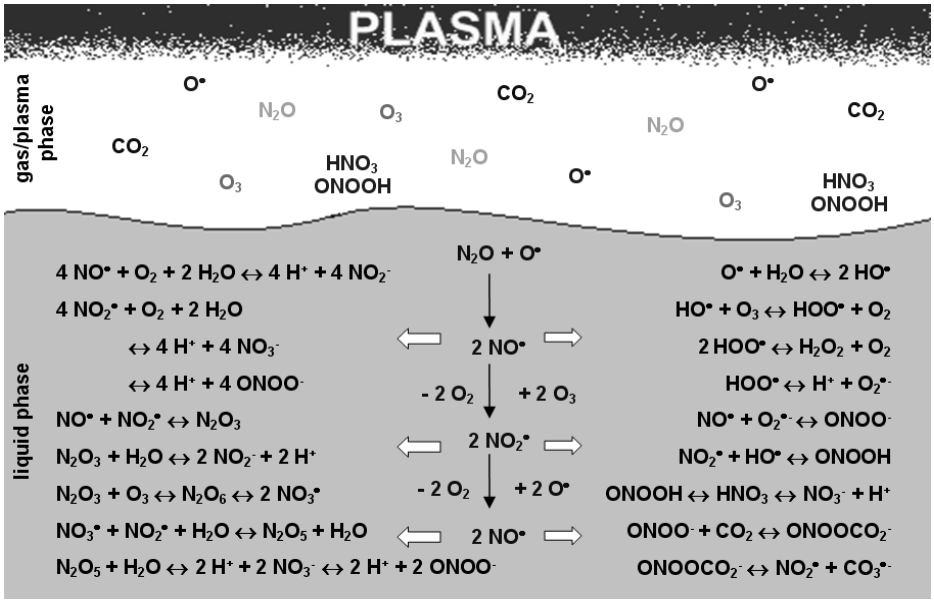

Fig. 2: Possible reaction channels of plasma/gas-liquid interactions

Acknowledgements

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Atmospheric pressure cold plasma processing of bioactive packaging applied directly to fresh fruits and vegetables

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Résumé

Described here is research for which the long-term goals are to improve public dietary choices and improve the economic return to fruit and vegetable producers through the development of processing techniques for healthier and safer fresh fruits and vegetables. Bioactive thin-film packaging can use a wide variety of immobilized molecules, antimicrobials, proteins, vitamins, yeasts and bacteria to improve many features of fresh fruits and vegetables. These bioactive species can become denatured (along with the fruits and vegetables being processed) at processing temperatures greater than 40ºC. Needed is a bioactive film deposition technique that is not dependent on thermal energy or harsh solvents. The central hypothesis in this research is that atmospheric pressure cold-plasma processing will provide a practical, non-thermal, non-toxic and non-allergenic means to apply bioactive thin films to fresh fruits and vegetables.

Introduction

Bioactive thin-film packaging applied directly to produce is a novel technology. Food safety and food quality for fresh fruits and vegetables will be improved by 1) extended shelf life; 2) improved nutrient content; 3) improved sensory qualities for the consumer; 4) introduction of antimicrobials; 5) improved gut health via probiotics and 6) extension of produce shelf life. Conventional thin film processes require heat or harsh solvents to form and attach the thin film thus they cannot apply active packaging films directly to fruits and vegetables without degrading the produce and/or degrading the functionality of the bioactive species that is to be immobilized in the matrix film. This two-page abstract describes research currently being conducted on this topic at Washington State University in Pullman, Washington. An early publication related to active packaging was a book edited 1.5 decades ago [1]. More recently flavonoids and phenolic acids have been identified as antimicrobial substances [2, 3] that have applications in active packing material that allows fresh fruits and vegetables to resist spoilage [4]. Cold plasma techniques have been used to treat the surfaces of nuts [5] and form plasma-polymerized films for controlled release of bioactive compounds [6]. It is reported in the literature that development of active packaging is hampered substantially by the thermolability of the active compounds which must be thermostable when entrapped in plastic films [7]. For example, ascorbic acid has been observed to take on a brown color in processes that used a temperature greater than 80°C [8].

Experimental setup

The existing experimental setup includes important modifications to hardware described recently in the literature [9]. In the work described here a streamer region is established between an array of 12 high voltage stainless steel needles and a grounded stainless steel torus. The reactor is shown in Figure 1. The carrier gas is argon with flow rate on the order of 100 standard cubic centimeters per minute (sccm) with surrogate precursor monomer molecules consisting of acetylene (flow rate on the order of 10 sccm.) The acetylene-based radicals flow downstream from the high voltage streamer region and form a thin film of plasma-polymerized acetylene on the substrate. Substrates include glass, highly ordered pyrolytic graphite (HOPG), mica and eventually fresh fruits and vegetables. Bioactive species will include probiotics, vitamins and antibacterial agents but the surrogate bioactive species used in the present work will be the antimicrobial molecule benzoic acid. Standard bioactive assessment techniques will be used to evaluate bioactive species functionality before and after processing [10, 11]. Thin film science diagnostic techniques that will be used to evaluate the bioactive films include the environmental scanning electron microscope (ESEM); atomic force microscopy (AFM); Fourier transform infrared spectroscopy (FTIR); x-ray photoelectron spectroscopy (XPS); the direct pull-off (DPO) method will measure film adhesion; BET isotherms will be used to measure specific surface area (porosity); a

thermo gravimetric analyzer (TGA) will measure thermal properties of the film; a contact angle meter will measure surface energy of the film; x-ray diffraction (XRD) will measure the crystalline structure within the film; and electrical permittivity of the film will be measured with impedance spectroscopy. Electrical energy input will be quantified by measuring with Rogowski coils corona current pulses. This energy input results in atomic processes such as ionization, bond scission, and excitation of atomic and molecular species to excited electronic states. Measuring this energy gives the team a technique with which to adjust the electrical energy input per unit of monomer mass. The bioactive species will be injected into the radical feed stream by a nebulizer. The bioactive species will be incident on the substrate along with radicals that are formed as the precursor molecules experience bond scission in the streamer tubes located in the cold plasma portion of the reactor. The bioactive species are immobilized in the growing matrix film via entrapment in pores or via covalent bonding to the matrix material.

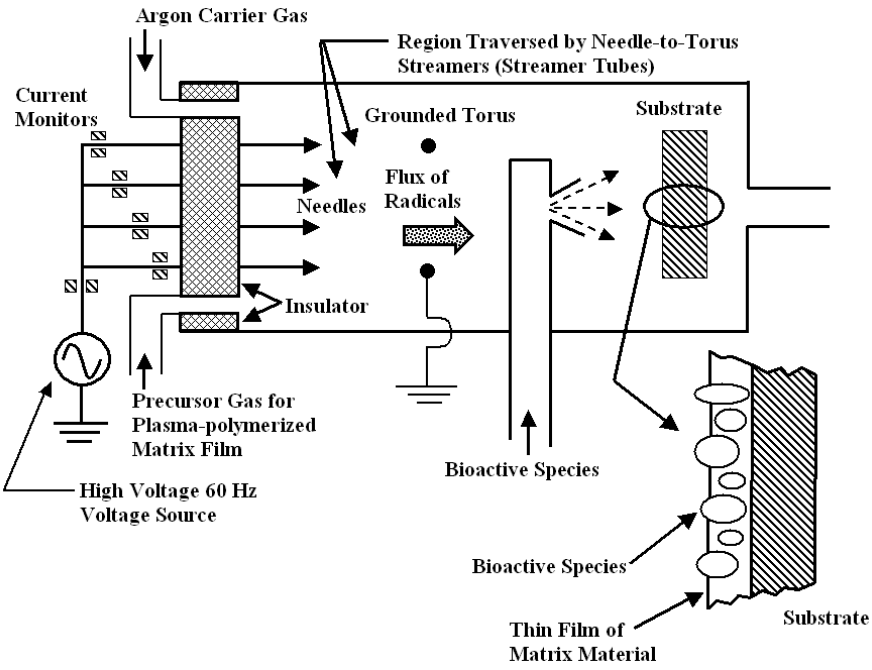

Fig. 1: This schematic diagram shows an artist's rendition of the atmospheric pressure cold plasma reactor used in this research. In the actual reactor design the carrier gas and precursor gas are mixed thoroughly before approaching the needle array. Not to scale.

Discussion and future work

An optimally designed reactor is one that generates appropriate radical species in the streamer tube regions and then facilitates transport of these radicals (without quenching of their dangling bonds) to the substrate with sufficiently high flux. Consumers, retailers, and producers will benefit from this technology as minimally processed fruits and vegetables become more nutritious, become more appetizing, contain fewer food-borne pathogens, and exhibit a longer shelf life. The effluent stream from this process can be nearly eliminated if the argon carrier gas is recycled. Nutritionally designed food systems that will make immediate use of this technology will promote consumer health via bioactive species that include probiotics [12], antioxidants [8], vitamins [8], and antimicrobials [13].

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Decontamination of *Bacillus subtilis* spores in a sealed package using a non-thermal plasma system

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Résumé

The safety of packaged food and medical devices is a major concern to consumers and government officials. Over the last century, numerous, alternative or complementary preservation technologies to classical (thermal) processing have been investigated. Non-thermal atmospheric plasma has been used effectively for surface decontamination. Apart from irradiation and heat, there is currently no technology which can inactivate microorganisms inside a sealed package. Using atmospheric plasma in a sealed package offers a significant breakthrough in food processing and safety technology.

A recent invention based on the principles of non-thermal, atmospheric plasma has shown the ability to reduce bacterial contamination inside a sealed package. The invention (PK-1) is based on a dielectric barrier discharge (DBD) with plate electrodes comprised of insulated conductors connected to a power unit with specifications of 18 kV @ 30 mA @ 60Hz. The package of the food is in contact with high voltage electrodes and provides dielectric resistance, thereby limiting current flow through the package and minimizing power requirements for treatment. Only 40-50 W of power are needed to ionize air inside a 4 L re-sealable plastic (LDPE) bag (Klockow and Keener, 2008). The process ionizes any gas within the electric field inside the packaged food or medical device utilizing the high voltage. Ionization can generate significant amounts of reactive molecules, including ozone concentrations above 1% in a few minutes. Only a few degrees increase in product surface temperature is observed for treatment times of minutes. Specific treatment times for targeted spore or bacterial reductions are dependent on product loading, packaging material, gas composition, and package/electrode configuration. The in-package ionization process has been demonstrated in a number common packaging materials including, cardboard, glass, LDPE, HDPE, PETE, polystyrene, rubber, Tygon, and others. A U.S. patent application has been filed on this technology. More recently, a new system design (PK-2) was built and has specifications of 130 kV at 20 mA @ 60Hz. The PK-2 system can ionize a sealed package of air with an electrode gap of 10 cm.

The objective of this study was to evaluate the PK-1 and PK-2 systems in the reduction of *Bacillus subtilis* spores using packages containing air and packages containing a MAP (modified atmosphere package) gas: 65% O_2/ 35% CO_2/ 5% N_2 treated inside and outside of a plasma field (gap space allowing), and examine the UV-Vis emission spectra for the ionization field of the PK-1 system as a point of reference in ionization characterization.

The experimental design consisted of the following parameters: 1) two voltage conditions: 13.5kV with 1.5cm electrode gap (PK-1) and 80kV with 3.2cm electrode gap (PK-2), 2) two treatment conditions: in and out of field of ionization, 3) PK-1 and PK-2 optimized treatment times for maximum ozone generation: 300 sec. (s) and 120 s, respectively, 4) temperature of 23°C, and 5) two package gas types: air and modified atmospheric package (MAP) gas: 65% O_2/ 35% CO_2/ 5% N_2 in a flexible low-density polyethylene package (LDPE plastic storage bag). Measurements included: 1) bacterial reductions of *Bacillus subtilis* var. niger (*B. atrophaeus*), 2) ozone, carbon monoxide, hydrogen peroxide concentrations using a portable Drager Measurement System, and 3) relative humidity. Spore strips (3.2 cm x 0.6 cm) containing Bacillus subtilis (1.5 x 10^6/strip) were loaded into sterile uncovered petri dishes and treated with ionization generated in packages using air or MAP gas blend. Samples were treated for 300 s (PK-1) or 120 s (PK-2) and stored at room temperature (23°C) for 24 h. Relative

humidity along with initial and final ozone, final carbon monoxide, and hydrogen peroxide concentrations were measured.

Results indicated initial and final relative humidity (RH) were 20% RH/23^{o}C and 30% RH/23^{o}C respectively. After 300 s of treatment (PK-1), ozone concentration was 3500 ppm (13.5kV/44W/1.5cm gap). After 120 s of treatment (PK-2), ozone concentrations were 10,000 ppm both in and out of ionization field (80kV/150W/3.2cm). Ozone concentrations measured < 0.5ppm or non-detect (ND) after 24 h from both systems. Carbon monoxide levels measured <10 ppm (PK-1), <20 ppm (in) and < 40 ppm (out) of ionization field of PK-2 after 24 h. Hydrogen peroxide levels measured zero or non-detect after 24 h from both systems. All treated samples showed reductions in *Bacillis* spores of greater than 2 $\log_{10}$ (PK-1) and 4 $\log_{10}$ (PK-2) after 24 h. Reductions were maintained without additional re-growth at 48h. The results indicate that the PK-1 and PK-2 ionization systems have the capacity to reduce *Bacillus subtilis* spores in an in-package treatment process.

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Plasma agents in water and surface decontamination

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Résumé

Bio-decontamination of water and surfaces contaminated by bacteria (*Salmonella typhimurium, Bacillus cereus, Escherichia coli*) was investigated in two types of positive DC discharges in atmospheric pressure air, in needle-to-plane geometry: the streamer corona and the transient spark with short high current pulses of limited energy. Both generate cold non-equilibrium plasma. Electro-spraying of the treated water through the needle electrode resulted in fast bio-decontamination. Experiments providing separation of various biocidal plasma agents, the emission spectra, oxidation stress measurements in the cell membranes, and chemical changes induced in the treated water helped better understanding of the plasma agents responsible for microbial inactivation. Radicals and reactive oxygen species seem to be dominant biocidal agents, although understanding plasma-induced water chemistry requires further research.

Introduction

In bio-decontamination by plasma, it is crucial to understand the role of various mechanisms involved. The significant mechanisms depend on the plasma composition (gas), temperature, treated microorganisms and the environment (air, water, surfaces, etc.). In atmospheric pressure plasmas, the major role is typically attributed to radicals and reactive oxygen species (ROS, e.g. OH, O, O_3) and to charged particles, especially O_2^- affecting the cell membranes. UV radiation plays a role only if photons in UV C germicide region (220-280 nm) or in vacuum UV are produced. In cold air discharges (corona, DBDs, pulsed discharges), UV C or VUV are usually not generated, so radicals and ROS are identified as the dominant bio-inactivation agents.

Results and discussion

We investigated the biocidal effects of two plasma sources in atmospheric air with water: positive DC streamer corona (SC) and a novel regime transient spark (TS). Despite DC applied voltage, these discharges have a pulsed character with nanosecond repetitive pulses and generate cold plasma. Their electrical parameters and emission spectra were documented in detail in our previous works [1-3].

The contaminated water flew directly through the stressed hollow needle electrode, and so through the plasma active zone in its proximity (Fig. 1), which substantially improved the volume efficiency compared to our previous set-ups for water treatment [3]. The effect of electrostatic spraying occurred when the high voltage was applied on the needle electrode. The temperature of the treated water did not change in SC and was increased by maximum 10 K in TS. The lethal heat effect to bacteria can be excluded.

We focused on the identification of the dominant plasma agents in bio-inactivation by coupling the electrical discharge characteristics (oscilloscopic measurements), their emission spectra (time-integrated and time-resolved), the chemical effects induced in water (measurements of pH, conductivity, H_2O_2, NO_2^- and NO_3^-) and their biocidal effects (thermostatic growth plate count method).

Depending on the initial conductivity of the treated water (we tested 1, 500, 1000 μS/cm, and physiologic solution ~14 mS/cm) and the plasma parameters (SC or TS, power, water flow rate, etc.), we observed a pH decrease from 5-7 down to 3-5 and an increase of conductivity (from 1 up to 1000, from 500 up to 1300 μS/cm). Nitrates (NO_3^-) reached concentration up to 2.5 mM, nitrites (NO_2^-) up to 116 μM and peroxides (H_2O_2) up to 500 μM. pH decrease is probably due to nitric acid formation, as is evident from high concentrations of the produced nitrates. However, additional tests showed that the nitric acid solution of the same pH does not lead to the same biocidal effects. In agreement with [5], it seems that acid environment in synergy with plasma agents leads to bacterial inactivation. In addition, we suppose an interaction of nitrites and peroxides at lowered pH; this has to be further studied with using buffers.

Unlike in [5], our preliminary tests show that even with PBS buffer that holds pH at 6.8 we obtain decontamination comparable to non-buffered plasma treatment.

Measurements of the oxidative stress induced in microbial cells (TBARS method [3-4]) enabled to further indicate their respective roles. TBARS concentration gain representing the oxidative stress induced in the cell membranes correlated with the bio-decontamination efficiency of SC and TS applied to the electro-sprayed water. The same samples were irradiated by biocidal UV C radiation (Hg lamp, 254 nm) for comparison. UV C induced almost no oxidative stress despite its efficiency was very high. This indicates that oxidations of cell membranes by ROS are important in microbial inactivation in SC and TS discharges. More ROS is linked with the higher efficiency [4].

Comparing direct with indirect plasma effects enables separation of various biocidal plasma agents. We compared direct SC and TS plasma treatment with 2 types of indirect exposure of contaminated agar surfaces: 1) through a grounded metal mesh letting but neutral active species to the agar; 2) through a quartz or MgF_2 windows allowing but UV radiation generated in the plasma to the agar. Interestingly, there was very little difference between the direct and the through-mesh indirect plasma treatment with both discharges, even at very treatment times as short as 5 s. This indicates that neutral reactive species are crucial even in the direct exposure. Exposure to the UV light only demonstrated no visible decontamination. This correlates with the emission spectra of SC and TS lacking any UV C or VUV. Similar effects of direct and indirect plasma treatment agree with the emission spectra, oxidation stress measurements and our previous findings [3-4].

Conclusion

Radicals and ROS were found the dominant biocidal agents in atmospheric air SC and TS discharges. However, plasma-induced water chemistry and its relation to bio-decontamination still arise many questions and so require further research.

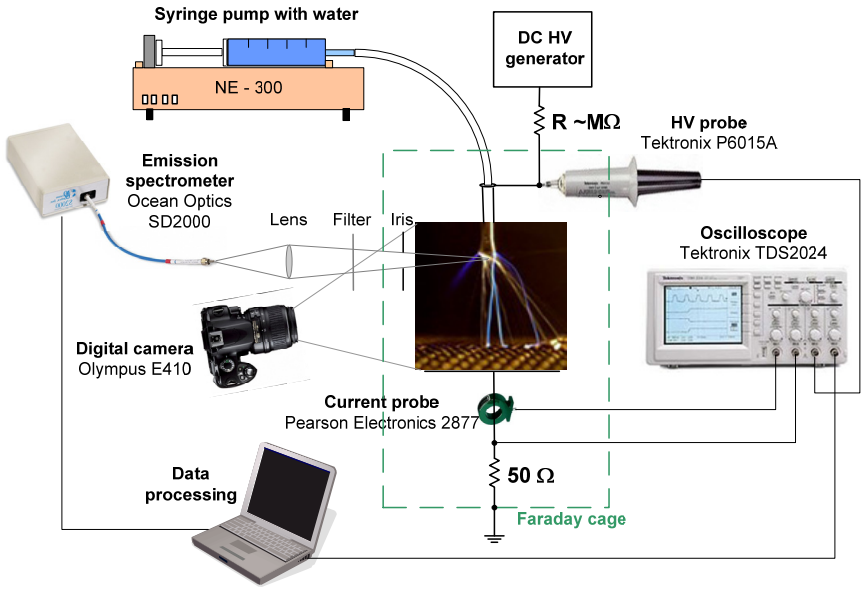

Fig. 1: Experimental set-up for DC discharges, with a high voltage hollow needle electrode enabling water flowing through the discharge zone and a plane or mesh electrode.

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Atmospheric pressure plasma jet interactions with plasmid DNA

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Résumé

The effect of a cold < 40°C radio frequency-driven atmospheric pressure plasma jet on plasmid DNA has been investigated. Gel electrophoresis was used to analyze the DNA forms post-treatment. The experimental data are fitted to a rate equation model that allows for quantitative determination of the rates of single and double strand break formation. The formation of double strand breaks correlates well with the atomic oxygen density. Taken with other measurements, this indicates that neutral components in the jet are effective in inducing double strand breaks.

Introduction

Cold atmospheric pressure plasmas offer a unique environment in plasma medicine, al- lowing treatment of soft materials, including bio-materials such as living tissues. Single plasma devices can be as small as 25 micrometers, thus approaching the size of a typical cell and allowing very precise treatment reducing damage to surrounding healthy living cells. Several bio-medical applications have already been identified, examples include bio- compatible implant coatings, skin diseases e.g. psoriasis, blood-coagulation, cancer treat- ments, tissue removal, and cosmetic treatments. In particular, the ability of non-thermal plasmas to inactivate micro-organisms has shown great promise for sterilization and in general decontamination on for example living tissues - wound healing, dental tooth caries, and on foods. Plasma interactions with living tissue should keep cell damage to a minimum. In general cell death should only be induced when necessary in a manner that the body can renew and repair itself i.e. apoptosis.

Little is known of the influence plasma has on DNA. While qualitative work is a good indicator, it is vital to quantitatively determine the nature of this influence before any potential application on living tissue can be realized. For applications such as skin treatments and wound healing it is vital that DNA damage is avoided. However, for cancer therapy controlled DNA damage may be desired. In particular formation of double-strand breaks is important as these are difficult for cells to repair. The motivation for investigations of DNA damage is two-fold. A fundamental understanding of plasma induced DNA damage for informing reliable risk benefit analysis [1]. Furthermore, DNA serves as a useful indicator with bio-molecules in general and the damage caused to it can be readily quantified. As an initial step it is essential to correlate direct plasma parameters with effects on bio-molecules.

In this study plasmid DNA in solution was exposed to the effluent of an rf atmospheric pressure plasma jet and simultaneously the absolute density of ground state atomic oxygen in the jet is measured. An effluent is emitted from the plasma bulk into ambient air and the investigations presented are performed a distance of 2 mm from the jet nozzle. The maximum gas temperature measured inside the plasma core using rotational bands of nitrogen is 75 C, while in the effluent applied to the DNA it is 40 C. The effluent of the plasma is allowed to interact with the plasmid DNA solution for varying treatment times and applied rf power. After exposure to the plasma jet gel electrophoresis was used to separate different DNA forms: supercoiled (SC), open circular (OC), and linear (LIN). For each set of conditions, the full study was repeated in triplicate with the standard error (SE) of each set of three measurements being used in subsequent fitting analysis. A system of rate equations governing damage in the plasmid was used to determine rates of each of the three forms.

The influence of UV emitted from the plasma was investigated by placing a $MgFl_2$ slide between the effluent and plasmid-DNA solution; UV from the plasma can penetrate the $MgFl_2$ slide while radicals are blocked. There was no evidence for single strand breaks (SSBs) or double strand breaks (DSBs) detected, even up to exposure times of 1 min. Exposure of the DNA to applied rf radiation only, without a plasma, and also gas flow only without a plasma were also investigated and again no influence was found.

The interactions at the plasma liquid interface are complex with a large variety of relevant species and a broad range of densities and particle fluxes. The discharge configuration is such that the electric field direction is perpendicular to the gas flow and plasma channel exit nozzle. This confines the charged species within the electrode gap inside the plasma bulk region. With no direct power input and very short mean free paths for charged species the effluent, upon interaction with the surrounding ambient air, is devoid of charge carriers. Neutral species, in particular reactive particles, and UV radiation dominate the effluent characteristics. Reactive oxygen species (ROS) and reactive nitrogen species (RNS), e.g. O, O_3, $O_2(a1\Delta g)$, OH, N, N_2, NO, are particularly known for their aggressive influence on biomolecules.

In order to investigate the role of radical damage to the plasmid-DNA, 10 mM Tris-EDTA – a substance known to be a much stronger radical scavenger than PBS – was used as a buffer solution. 100 mM Tris-EDTA mimics the radical scavenging environment found in cells in protecting DNA from damage. However the damage incurred to the DNA in 10 mM Tris-EDTA is greatly reduced compared to that in water or PBS. From this we can conclude that radicals play an important role in DNA damage.

Figure 1 shows the rates for SSB and DSB formation as a function of absolute atomic oxygen density measured in the core of the plasma bulk. It should be emphasized that there may be other plasma species or a combination of two or more species either in the plasma or induced downstream products causing DNA damage. However, as a starting point atomic oxygen has been identified as a relevant species, either directly or indirectly, correlated to DNA damage.

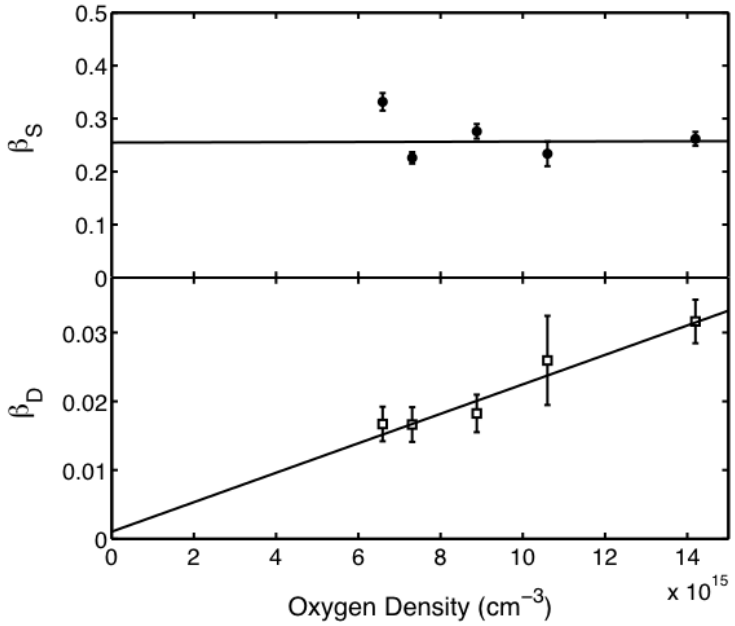

Fig. 1: Rate of single and double strand breaks of the plasmid DNA in 10 mM PBS as a function of absolute atomic oxygen density. The error bars are the uncertainties in the parameters βS and βD as derived from the fitting process. The lines are best-fit lines derived from a weighted linear least squares fit to each of the data sets.

The slope of this line then hints to there being a component or components in the plasma which are very effective at producing DSBs and that these components are well correlated with the atomic oxygen density. It is however not fully conclusive to assume that it is the atomic oxygen itself which is responsible for DSB production. The density of other species within the plasma may also correlate with atomic oxygen. In contrast, the rate of SSBs shows no evidence of dependence on atomic oxygen density. It is very unusual to find a means to form DSBs that does not also form SSBs. Since β_S is typically two orders of magnitude greater than β_D it is not certain this is the case here in spite of the lack of correlation between β_S and atomic oxygen density, since the effect of this species on β_S may be masked by other components which give rise to SSBs. Ozone and singlet delta oxygen densities were also measured and do not show a direct correlation to either single or double strand break rates.

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DNA oxidation by reactive oxygen species produced by atmospheric pressure microplasmas

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Résumé

Arrays of microcathode sustained discharges (MCSD) have been developed for the production of high fluxes of singlet delta oxygen (SDO) and ozone (O_3) at atmospheric pressure. SDO and O_3 densities higher than, respectively, 10^{17} and 10^{16} cm^{-3} have been efficiently produced and transported over distances longer than 50 cm. These arrays of MCSD have been optimized to supply well-quantified and tunable fluxes of either SDO or O_3. This plasma source has been found to be very useful for examining the reactivity of these reactive oxygen species with biological components. Experiments were performed strongly indicating that both SDO and O_3 are able to oxidize DNA, originating great damages in DNA such as double-strand breaks and base oxidation. It has been observed that while all bases of DNA are almost indifferently and quite effectively oxidized by O_3, SDO reacts mainly with guanine.

Introduction

Reactive oxygen species (ROS) are well known to play an important role in several biological systems, and generate oxidative damage to a variety of cellular components [1]. Among others, deoxyribonucleic acid (DNA) is of particular importance, due to its key role in cell survival and reproduction. Fundamental studies examining the cellular components targeted by different ROS generated in low-temperature plasmas, and the modifications induced by those interactions, are, thus, quite interesting and very promising for biomedical applications. In this context, we have developed arrays of microcathode sustained discharges (MCSD) for the production of ROS at atmospheric pressure. The remarkable stability of MCSD has allowed us to operate DC glow discharges in He/O_2 mixtures, free from the glow-to-arc transition, at high gas pressure, with low values of the reduced electric field (5–10 Td) and of the gas temperature (300–400 K) [2]. As a result, large amounts of singlet delta oxygen (SDO) and ozone (O_3) have been obtained at atmospheric pressure. In fact, SDO densities higher than 10^{17} cm^{-3} have been efficiently produced and transported over distances longer than 50 cm, providing SDO fluxes greater than 100 mmol/h. Furthermore, O_3 densities up to 10^{16} cm^{-3} have also been obtained. Besides that, the density ratio of SDO to O_3 can be finely and easily tuned in the range 10^{-3}–10^{+5} [3]. As so, these arrays of MCSD, by allowing a controlled production of either SDO or O_3 at atmospheric pressure, are ideal tools for studying in details the reactivity of these ROS towards biological components, notably DNA.

In order to study the reactivity of SDO and O_3 towards DNA, we have made interact aqueous solutions of DNA with a gas flow of either SDO or O_3, as previously described in details in [4]. Oxidation of DNA has been performed for different ROS flows and times of interaction. While the damages to the DNA backbone were analyzed by agarose gel electrophoresis [5], the products of oxidation were detected and quantified using the accurate and sensitive high performance liquid chromatography tandem mass spectrometry method (HPLC-EIS-MS/MS) [6].

Results and discussion

In the present work, preliminary results of experiments concerning the use of arrays of MCSD as a plasma source for biomedical applications are presented and discussed. As illustrated in Figures 1 and 2, the experiments that have been conducted strongly indicate that SDO and O_3 are able to oxidize DNA, originating great damages in DNA such as double-strand breaks and base oxidation. It has been observed that while all bases of DNA are almost indifferently and quite effectively oxidized by O_3, SDO reacts mainly with guanine. Moreover, O_3 seems to be much more effective on oxidizing DNA. Indeed, as one can see in Figure 1, double-strand breaks only occurred when using gas flows of O_3. Besides that, as exemplified in Figure 2, the amount of modified bases is also much higher when making O_3 molecules

interact with the DNA solutions, compared to the use of gas flows of SDO. The enhancing effect of heavy water (D_2O) has been used to confirm the SDO-mediated DNA oxidation. When using heavy water, not only the same trends have been observed as when using H_2O, but also from 5 to 30 times more damages were induced (cf. Figure 2), correlating, therefore, the oxidized nucleosides formation to the presence of SDO. In fact, the SDO lifetime in D_2O is about 10 times longer than in H_2O [7].

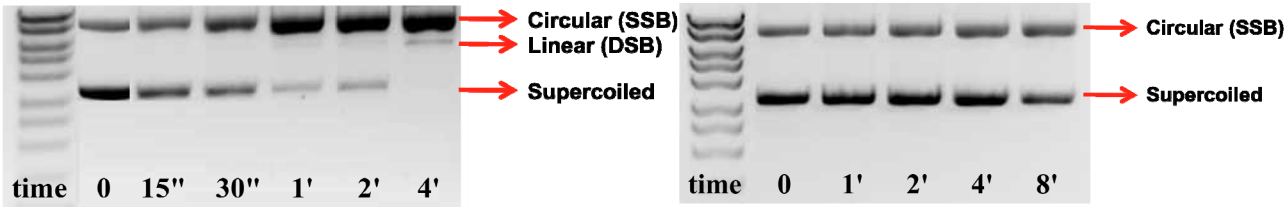

Fig. 1: Digital photographs of the agarose gel showing typical separation of DNA populations of different conformation subsequent to different times of interaction between the aqueous solution of plasmid DNA and an afterglow gas flow of O_3 (left) and SDO (right).

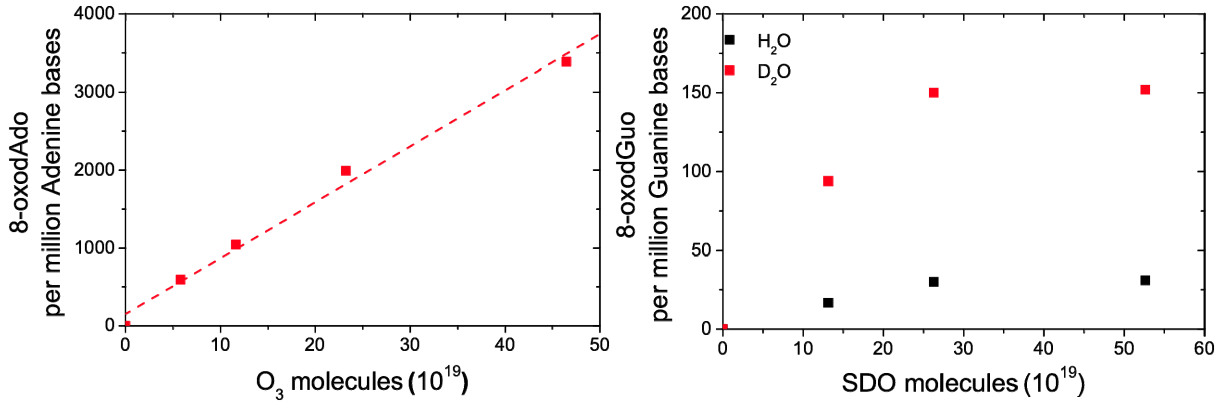

Fig. 2: Evolution, versus the O_3 (left) and the SDO (right) molecules reaching the DNA solution during given times of oxidation, of the quantity per million bases of the products of oxidation of adenine (left) and guanine (right) resulting from the degradation of different aqueous solutions of DNA: black squares = H_2O, and red squares = D_2O.

The results that have been obtained, even if preliminary, are very significant and demonstrate that arrays of MCSD are a quite promising plasma source, being very suitable and useful tools for biological studies, and are, thus, likely to lead to new biomedical applications. In fact, in the context of the new field of Plasma Medicine, our plasma source is unique. Indeed, contrary to the other available sources of reactive oxygen species, our arrays of MCSD are able to supply well-quantified and tunable fluxes of either SDO or O_3. Nevertheless, there are still many open questions on the reactivity of ROS with DNA. For a better understanding of the mechanism of ROS-mediated oxidation of DNA, efforts are to be made to gain further insights into the chemistry of the liquid phase. A more detailed study on this subject is currently in progress.

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Investigations of bacterial inactivation and DNA fragmentation induced by flowing humid argon post-discharge

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Résumé

Bio-contaminated surfaces were exposed to an atmospheric flowing post-discharge, i.e. without direct contact of the plasma with the surface. The non-thermal plasma source was a dielectric barrier discharge in a cylinder-to-cylinder geometry. Using humid argon as a feed gas, a reduction of 6 orders of magnitude of survivors could be obtained for *Escherichia coli*. An investigation of the bacterial inactivation mechanisms during the plasma induced treatment was conducted. For this purpose, DNA (genomic DNA in aqueous solution) degradation by the plasma process was studied, assuming that the bacterial inactivation is obtained when the bacterial DNA is fragmented. According to the operating conditions (feed gas flow rate and discharge input power), DNA fragmentation was evaluated in correlation with aqueous phase hydrogen peroxide concentration measurements. It appears that hydrogen peroxide is not the only responsible factor for DNA fragmentation.

Introduction

Non-thermal plasma technologies have been heavily investigated during the last decade for surface decontamination of thermally sensitive materials. Several techniques were studied with different excitation sources operating in different gases from 1 Torr to atmospheric pressure [1-4]. The dielectric barrier discharge (DBD) process under investigation operates at atmospheric pressure in humid argon. The contaminated surfaces to be treated are exposed to the flowing post-discharge, i.e. to the discharge products, without direct contact of the plasma with the surface. Assuming that the bacterial inactivation is obtained when DNA damage occurs, investigations of a model planktonic microorganism inactivation and DNA molecule fragmentation were conducted during plasma treatment in separate experiments.

Experimental

For all experiments, the DBD reactor (Fig.1) consisted of a stainless steel rod (2 mm diameter) centered in a dielectric tube (6 mm and 3 mm external and internal diameters respectively) externally covered with a 24 mm length copper tape connected to ground. The inner electrode was connected to an AC (30kHz) high voltage power supply (2.5-10W discharge input power). The DBD reactor was fed with humid argon (RH ≥ 95% at room temperature, 0.5-2L/min). The outlet of the discharge tube was connected to the treatment vessel in which biological samples were exposed to the flowing post-discharge. More precisely, biological samples were placed 5 mm from the tube outlet. The bacteria were treated in 10µL (20%Vol. LB in distilled water) droplets (~10^8 bacteria/mL *Escherichia coli* DH10B strain) spotted on sterile glass slides. Once plasma treatment achieved, bacterial inactivation was evaluated by direct colony counting after collection and incubation (24h at 37°C).

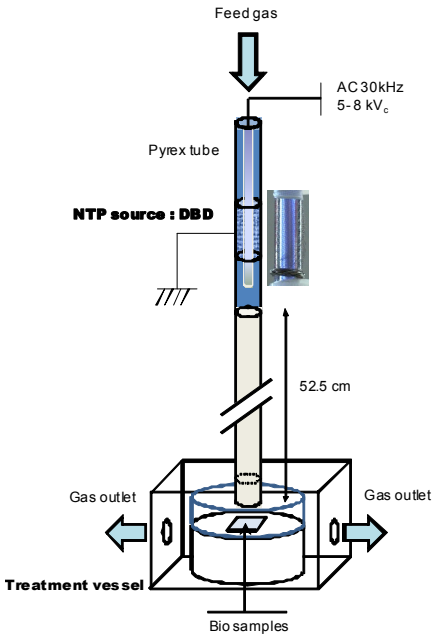

Fig. 1: DBD source and bio sample treatment device

DNA solutions were treated in 10 µL distilled water droplets (1mg/mL) spotted on sterile glass slides. As a model DNA, genomic DNA (salmon sperm) was used. After treatment, agarose gel electrophoresis was used to separate DNA molecules according to their molecular weight and 3-D structure.

Hydrogen peroxide is, with oxygen and hydrogen, an end product of water dissociation by excited argon atoms. Its high Henry's Law constant (10^5 M/atm) and oxidative properties lead to a possible

decontamination effect. Hydrogen peroxide concentration was measured in 10 µL distilled water droplets submitted to the plasma post-discharge, using a colorimetric method [2].

Results

After a 10 min exposure to the DBD post-discharge (2.5W - 0.5L/min humid argon) of 10 µL water droplets, 10 µL bacteria suspension droplets and 10 µL DNA solution droplets, the following results were respectively obtained: (i) 150ppm (w/w) hydrogen peroxide concentration, (ii) reduction of 3 orders of magnitude of survivors (from 10^7 to 10^4 *E. coli*), (iii) fragmentation of the genomic DNA (Fig. 2: lanes 1&2 to be compared to the control in lane 3). The same quantity of DNA was incubated during 10 min in H_2O_2 solutions with increasing concentrations from 100ppm to 250ppm (lanes 10, 12, 14, 16 in Fig.2). No DNA fragmentation was observed, demonstrating the low activity of hydrogen peroxide for DNA fragmentation and the role played by other oxidative species, e.g. OH and/or HO_2. Note also that an increase in discharge input power led to a higher DNA fragmentation (see migration band enlarged and shifted toward low molecular weight – bottom of Fig. 2 lanes 4,5,7,8).

In order to reduce the transfer time of short lived species from the DBD source to the surface to be treated, the total flow rate was increased and in the case of some bacterial inactivation experiments, a 525mm PFA tube (6mm ID) could be inserted between the outlet of the DBD tube and the biological samples, in order to estimate the decontamination efficiency loss according to transfer time of the activated species from the plasma source. Results are presented in Fig.3 for *E coli* inactivation. A reduction of at least five orders of magnitude was achieved within 10 min.

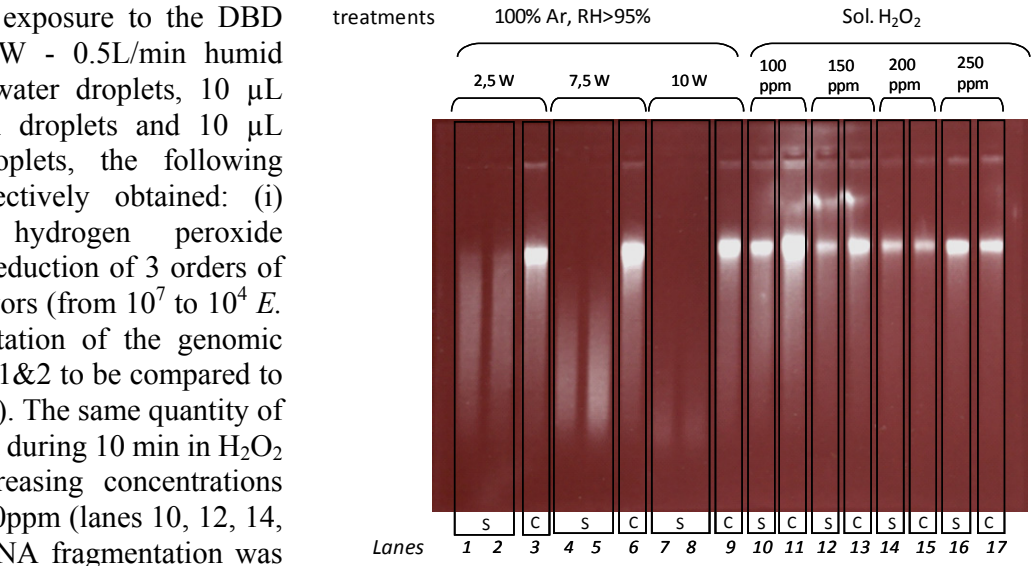

Fig. 2: Genomic DNA agarose gel electrophoresis after different treatment conditions. Lanes 1 to 9: 10 min. plasma treatment (2.5W - 0.5L/min humid argon). Lanes 10 to 17: 10 min incubation in H_2O_2 sol. (S = samples, C = controls, migration from the top to the bottom, .i.e. from high molecular weight to low molecular weight)

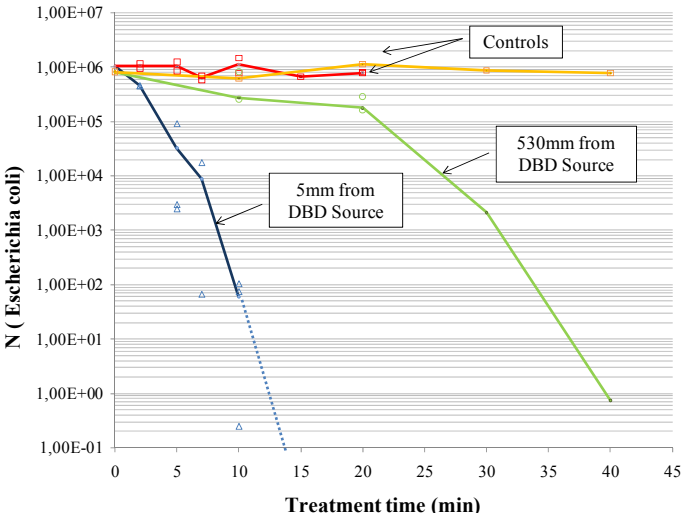

Fig.3: *E. coli* survivors vs. exposure duration to DBD flowing post-discharge (2.5W - 2L/min humid argon)

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Helium atmospheric pressure plasma jet: Diagnostics and application for burned wounds healing

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Résumé

A new field of plasma applications developed in the last years, with the short title plasma medicine, focused the attention of many peoples from plasma community on biology and medicine. Subjects that involve plasma physics and technology (e.g. living tissue treatment or wound healing, cancer cell apoptosis, blood coagulation, sterilization and decontamination) are nowadays in study in many laboratories. In this paper we present results on optical and electrical diagnosis of a helium atmospheric pressure plasma jet. This type of plasma jet was used for modification of wound healing process and we obtained an acceleration of the process with 50% against control group. Data from biochemistry and histology tests are presented and commented.

Introduction

The growing importance of plasma technologies in medicine, biology and health care is a fact reflected by the increasing number of scientific publications or reports on this subject [1]. For many applications low pressure plasma sources have several drawbacks and their potential use in medicine is limited. In contrast, atmospheric pressure plasma sources are presented in literature as good candidates for medical use. They are labeled in different ways, well known names being plasma needle, plasma pencil, plasma gun or atmospheric pressure plasma jet (APPJ). Plasma is generated in these sources using various geometries and working gases, using the principles of corona discharge, dielectric barrier discharge and microdischarges. Parameters of these plasma sources (e.g. temperature and concentration of charged species, gas temperature, concentration and distribution of reactive species) are spread over large domains of values. Having this in mind, standardization of operational parameters of atmospheric pressure plasma sources represents a necessity before any large medical use and remains an open challenge. Parameters like maximum current value, charge, power density, repetition rate, applied voltage can be used to achieve this goal.

Helium APPJ

The helium APPJ, designed in our laboratory, is generated in a dielectric barrier discharge configuration. Aluminum tape electrodes are wrapped on a quartz tube, 4 mm inner diameter and separated by 10 mm space. Helium is flowing trough this quartz tube with a flow rate of few liters/min. High voltage monopolar square pulses are applied on powered electrode with a repetition rate between 0.5 to 4 kHz, with variable amplitude and pulse width. The signals of discharge current and applied voltage are monitored using probes and digital scope (TDS5034, Tektronix). Images of our plasma jet in the nanoseconds range are obtained using a Hamamatsu C8484-05G camera (Fig. 1).

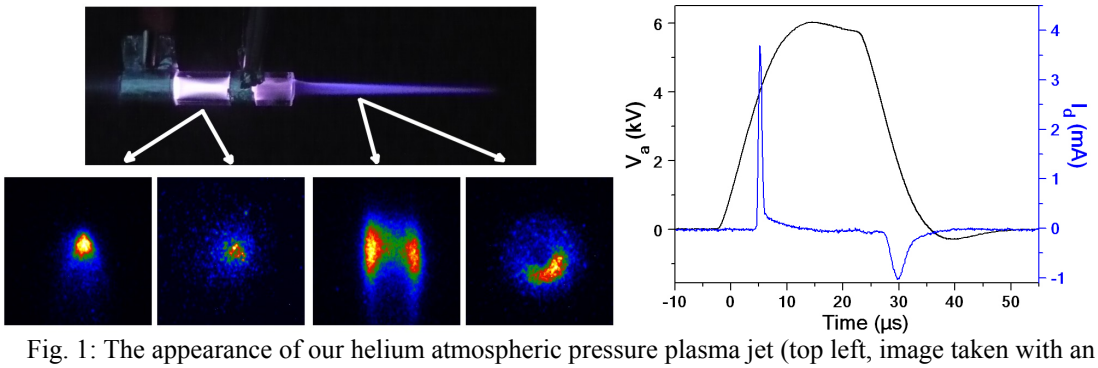

Fig. 1: The appearance of our helium atmospheric pressure plasma jet (top left, image taken with an usual camera and bottom left, 30 ns exposure time images taken with an ICCD camera, side view and on-axis view) and typical voltage-current waveforms.

The length of plasma jet is mainly controlled by the helium flow rate, having values from few mm to around 5 cm (flow rate: 4 L/min). The current is characterized by two sharp peaks, which correspond to primary and secondary discharges. The amplitude of these peaks, as well the values of charge, peak power and average pulse power are function of the amplitude of the applied voltage pulse, on its width and the repetition rate. For an amplitude of the applied voltage pulse from 3 to 8 kV (width 30 μs, repetition rate of 2 kHz, flow rate 3 L/min), the primary current peak value increases from 1 mA to 2.5 mA, the charge from 1.8 nC to 4.7 nC, the power peak from to 3 to 20 W and the average pulse power from 0.5 to 2.5 W.

Regarding the propagation mechanism we found for this type of plasma jet two very different types of plasma bullets: homogenous ones inside the quartz tube and non-homogenous ones outside the tube, where the air surrounds the plasma jet. In cross section, the light emitted from these bullets has a non-homogenous intensity [2]. The speeds of these luminous structures are as follow: the homogenous bullet starts with a speed around 6.5 km/s and the speed increases in the vicinity the ground electrode to 22 km/s, while the non-homogenous bullets present at the beginning a speed of 6 km/s and increases up to 14 km/s.

Application for wound healing

The above presented helium atmospheric pressure plasma jet was used to compare spontaneous reepithelization versus plasma-assisted reepithelization (40s daily treatment). Reproducible burned wounds were produced on the back skin of Wistar rats. The protocol for the animal experiments has been approved by the Ethics Committee of „Grigore T. Popa" University of Iasi. Hematological, biochemical and histological data were measured at different time intervals to monitor the evolution of systemic and local effects.

After 21 days, the hematological and biochemical data showed no differences between normal values compared to control group. However, the histological images showed that subjacent dermis regenerates for the plasma treated group of wounds (Fig. 2). For these plasma treated wounds, it is worth to mention that we found significant differences in the values of local oxidative stress markers.

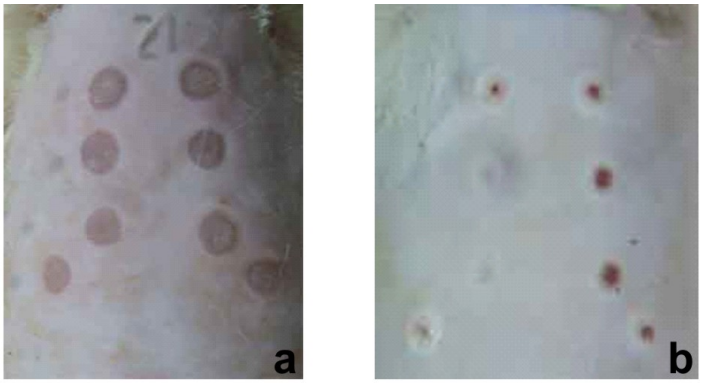

Fig. 2: Typical clinical appearance of (a) fresh wounds and (b) after 21 days: left side, daily plasma treatment for 40s and right side control group.

Conclusion and perspectives

Helium atmospheric pressure plasma jet represents a good candidate of plasma source for medical applications. Its operation is reproducible and can be standardized, while the economic aspects can be adjusted to obtain a profitable system. This type of plasma jet was used to accelerate the wound healing process and the results are promising.

For a better understanding of mechanism that are behind the benefic effects in plasma medicine, experiments regarding plasma effects on supramolecular biological systems like proteins are carried now our group. Plasma effects on protein structure and structure to function relationships are investigated. Time dependence of plasma effects on biological molecules is also an important part of our studies.

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SDBD plasma jet for skin disinfection

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Résumé

A consortium consisting of the research institute TNO, the medical university and hospital St Radboud and two industrial enterprises is working on a non-thermal plasma treatment method for skin and wound disinfection. The group is seeking for cooperation, in particular in the field validation methods and potential standardization for plasma based disinfection procedures. The present paper briefly presents the technical progress in plasma source development together with initial microbiological data obtained.

Introduction

Hand hygiene is an important topic in hospitals and medical practices as it reduces transmission of infectious diseases. Frequently applied disinfection of hands and underarms by available alcohol based disinfectants is time demanding, results in a too dry skin and often causes skin irritation. This research project is aimed at a standardized plasma-based hand disinfection procedure in accordance with basic disinfection requirements, without drawbacks of current methods, to be safely applicable and available at affordable cost.

Plasma produced in ambient or nitrogen enriched humid air produces a variety of reactive oxygen- and nitrogen species (ROS, RNS) such as the superoxide anion O_2^- and nitric oxide NO. Directed to a wet surface, transferred plasma species create secondary reactive products in solution such as peroxides. One must be aware of the fact that in the biomedical research field those chemical reactive species are not only known from other types of sources (UV, ionizing radiation) but even more that many of them are produced by cells themselves. Enzymatic production of reactive oxygen species naturally occurs in cells and cell membranes. Selected ROS and RNS may be subject to transmission through cell membranes, and may have functions in cellular signaling essential for the proper development and proliferation of cells, wound response and healing.

Different types of plasma sources which are under investigation for medical application can be classified according various criteria such as electric energy coupling modes (microwave, dielectric barrier and arc discharges), gas composition and flow as well as 'location' of the ionizing high electric field region either in vicinity of the skin/wound, at remote distance from it or a combination of both. Though plasma generation at the skin surface is possible even at low voltage potential, the remote generation of reactive plasma species is preferable for reasons of increased safety and reduced dependence of skin conductivity and morphology. In plasma jets fast transport of neutral and charged reactive species to the substrate is achieved with a forced flow. There is growing recognition that at least for N_2-O_2 mixtures, transport of ROS and RNS is of critical importance while UV radiation and electric field induced electroporation play a minor role in microbial inactivation at the remote surface [1]. However, influence of composition, temperature and flow of the plasma produced gas on skin/wound disinfection still needs further investigation in order to standardize the treatment.

Plasma source characterisation

A plasma jet source has been constructed which uses an alumina ceramic tube having an inner temperature conditioned high voltage conductor and is positioned at adjustable distance from the surface to be treated. Two parallel flattened surfaces of this tube form a thin gas space with the adjacent exterior electrode. The exterior electrodes have protruding ribs touching the ceramic and serving as starting points for a large number density of discharges on the ceramic surface. The surface discharges are created in volume spaces of 5x5xd mm^3. Different structures allow selecting the thickness d as 100, 200 or 500 micron. Figure 1 shows different aspects of the system which includes a movable substrate table which can be moved as adjustable speeds below the plasma jet source and a liquid spray unit.

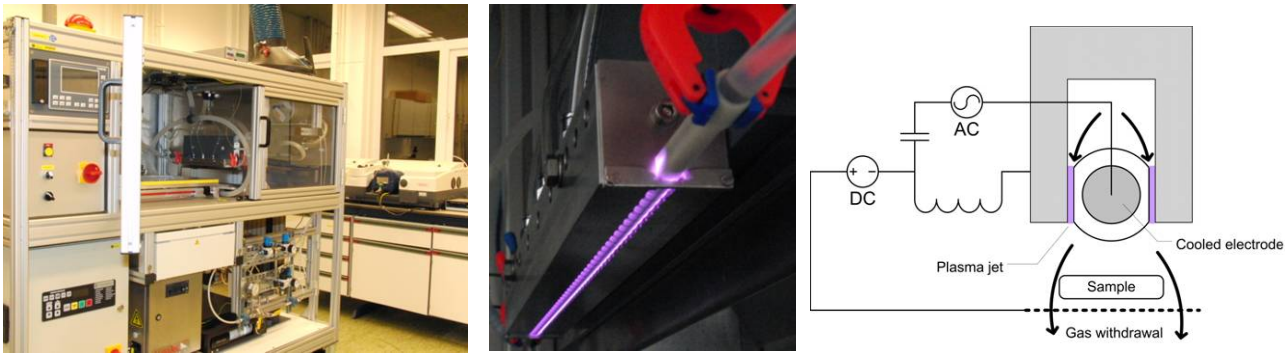

Fig. 1: (left) Test unit with movable substrate table, spray and gas withdrawal systems, (centre) tubular cooled SDBD plasma jet source, (right) electrode cross section with unipolar charging circuit.

This plasma jet source is characterised by fast transport of reactive plasma species in a modest gas flow, effective temperature control of the ceramic allowing a high power density (up to 1000 Watt/300 mm tube length), gas temperature controlled ratio ROS/RNS and regulated electrical charging using asymmetric pulse amplitudes or a superimposed DC+AC potential (WO2008082297, WO2010047593). Using the finite element software package Comsol the temperature and flow field have been calculated as shown in Figure 2.

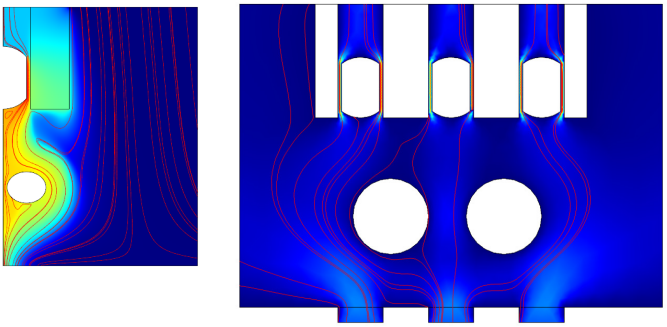

Fig. 2: Examples of FEM calculations showing the effects of gas transport around objects, (left) temperature distribution resulting from a single electrode structure (red=330K, yellow = 310K), (right) gas flow field using 3 parallel electrode structures. Applied flow conditions are 3 L/min/cm, total gas withdrawal flow is 2x the gas supply flow. The calculations show the importance of parallel jets and gas withdrawal for effective treatment of large shapes such as a human hands and underarms.

Microbiological inactivation

E. coli and *B. globigii* have been treated in dry form on glass slides (contaminated area = 20x35 mm^2). First results indicate that ~20 sec treatment at 10 mm distance from the source nozzle results in a significant reduction of colony forming units from *E. coli.* A thin film of distilled water (~0.1 mg/cm^2) causes a dramatic further increase of the inactivation efficiency. Replacing water with a 5% chloroxylenol solution is shown to cause a small but significant synergetic effect. A one log decrease of *B. globigii* spores is observed in this case as well.

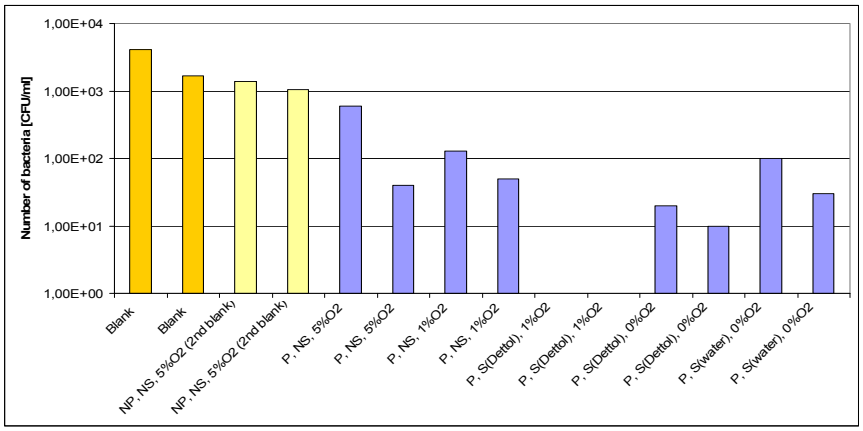

Fig. 3: Microbiological inactivation data for *E. coli* treated by a 500W plasma jet with 2 mm/s sample movement. P, NP design plasma and no plasma treatment. S, NS design spray and no spray treatment. The percentage oxygen concentration in nitrogen has been varied. Gas through the jet is heated from 25 to 40 deg ^{o}C (Neoptix optical fibre system at the nozzle outlet) while the cooling water temperature is increased from 20 to 31 ^{o}C at 25 L/h (~320 W).

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Synergistic effects of tissue tolerable plasma and polihexanide to promote healing of chronic wounds - in vivo and in vitro results

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Résumé

The effects of combined plasma - polihexanide (PHMB) application on cell integrity, cytotoxicity and its irritative and inflammative potential were tested *in vitro* and *in vivo* in two dogs. The combined application showed synergistic effects in the treatment of chronic wounds. Tissue tolerable plasma works as promoter for wound healing and can be beneficially combined with the antiseptic PHMB polihexanide to avoid bacterial recolonization.

Introduction

A chronic wound is characterized by a chronic inflammation and critical bacterial colonization which prevent it from healing in an orderly set of stages. Only a small number of planctonic bacteria are sufficient to adhere to the wound surface; to multiply and to develop into microcolonies over short time, which in turn form larger aggregates known as biofilms [1, 2]. In this context, two therapeutic problems have to be solved: cleaning of the critically colonized or biofilm loaded wound surface and turning the persisting chronic inflammation into an acute one to initiate the healing process. In recent time, tissue tolerable plasma (TTP) was distinguished to enhance wound healing [3]. We were able to show in the modified HET-CAM that plasma application can induce aseptic inflammations, which are suitable to modulate chronic inflammations [4, 5]. The antimicrobial effects of TPP are well described [6]. Still, the disadvantage of TPP is that the antimicrobial effect is immediate only and does not last long enough to prevent bacterial recolonization. We therefore combined plasma treatment with the antimicrobial substance polihexanide [7] *in vitro* and *in vivo* to add a sustained effect. Polihexanide is a first line substance for treatment of chronic wound due to its broad antimicrobial spectrum, good tissue tolerability and its ability to bind to the organic matrix. Recently, a stimulating effect of polihexanide was demonstrated [8]. We used the Reconstructed Epidermis (RE) to assess cytotoxicity effects and their reversibility (MTT – method) of combined TPP - polihexanide treatment on NHEK (normal human epidermal keratinocytes) - cells. The integrity of the RE was determined by means of the Trans-Epithelial Electrical Resistance (TEER). In the modified HET-CAM, we assessed if the combined treatment led to pronounced irritation or inflammation. The positive results from the *in vitro* assays encouraged us to test the combined treatment in two dogs. Both dogs suffered of chronic wounds for several months, conventional therapies failed. The treatment was well tolerated by both animals. In one case, the treatment led to a complete healing after 11 weeks, which was particularly remarkable because the treatment with TPP or polihexanide alone did not led to healing. This supports the presumption, that TPP and polihexanide have synergistic effects in promoting healing of chronic wounds. In the second case, the treatment was complicated by constant licking and irritation of the wound by the dog, but the healing progressed after applying a ruff and the wound has improved since then. To conclude, the applied methods (modified HET-CAM for irritation - and inflammation potential; RE for the assessment of the cytotoxicity and integrity) are suitable for screening plasma sources and parameters for medical applications alone and in combination with polihexanide. The combined TPP-polihexanide treatment is a promising option for the treatment of chronic wounds.

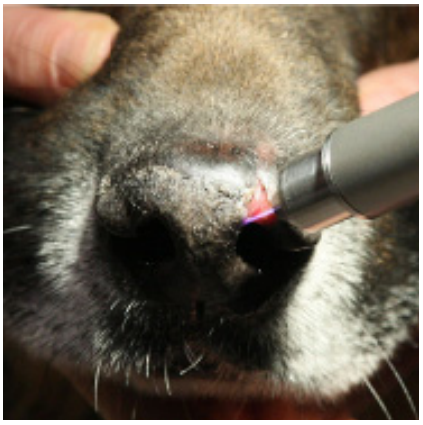

Fig. 1: Plasma treatment of the wound.

Fig. 2: State of the wound, 11 weeks after combined plasma-polihexanide therapy.

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Cold atmospheric plasma for clinical purposes – promising results in patients and future applications

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Résumé

Infected chronic wounds are both socioeconomic and medical problem. Cold atmospheric plasma (CAP) has already proven its efficacy in killing bacteria on agar plates but also in a first prospective randomized controlled trial in patients. As an add-on therapy CAPs proofed a highly significant decrease in bacterial load in 5 min plasma-treated wounds (34%, $p<10^{-6}$, n=291, 36 patients) in comparison with wounds that received only standard wound care. This reduction is found in all kinds of germs, even multiresistant ones. Just as 2 min as well (40%, $p<0.016$, n=70, 14 patients). The treatment is very well tolerated and no side effects occurred until now (in total more than 2000 treatments in over 220 patients). The results of this study revealed the potential of atmospheric argon plasma treatment as a new approach to kill bacteria in terms of mutiresistancy.

The observed bactericidal effect of plasma therapy relies on the synergy of reactive oxygen and nitrogen species, charged particles, electric fields, and UVR. The combination of these biologically active components makes plasma an efficient tool for fighting bacteria.

With the same CAP device other dermatologic diseases were treated successfully, e.g. Hailey-Hailey disease. Otherwise CAPs failed in some diseases and revealed their limitations.

New plasma devices using surrounding ambient air have not only greater bactericidal but also virucidal properties. These devices may herald a new era in public, personal, pet, and food hygiene, same as in decontamination. Investigations of human compatibility are promising.

Non-equilibrium air plasma for wound bleeding control

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Résumé

A low temperature non-equilibrium air plasma spray is tested as a blood coagulator. Emission spectroscopy of the plasma effluent indicates that it carries abundant reactive atomic oxygen (RAO), which can activate erythrocyte – platelet interactions to enhance blood coagulation for plug formation. Tests of the device for bleeding control were performed on pigs. Four types of wounds, straight cut and cross cut in the ham area, and a hole in a saphenous vein and in an artery of an ear were examined. The results were that this plasma torch shortened the bleeding time, for the first three types of wounds, from about 3 minutes to 18 seconds, from about 4 minutes to 25 seconds, and from 88 seconds to 15 seconds, respectively, as well as effectively clogged the hole in the artery to stop bleeding. The tests indicate that RAO can penetrate through the skin surrounding the wound to block capillary blood flow to the wound, making it fast to stop bleeding.

Bleeding, even from an external hemorrhage, may be life threatening if it is not treated swiftly [1]. Most cases occur under emergency situations. New methods and devices which can effectively stop bleeding could save the life of an injured person, especially in battlefield situations. Chen *et al*. [2] and Kuo *et al*. [3] showed that a low temperature non-equilibrium air plasma spray [4] could clot anti-coagulated whole blood samples in less than 20 seconds, which is much less than 30 minutes for an untreated sample to reach complete coagulation. It was also found via emission spectroscopy that this plasma spray carries abundant reactive atomic oxygen (RAO) in its plasma effluent [5]. Activation of erythrocyte – platelet interactions by RAO to trigger blood coagulation was suggested as a plausible coagulation mechanism.

However, those experimental results performed under well-controlled in-vitro conditions may not describe what occurs in the much more complicated in-vivo environment. In the present work, we use pigs as the animal model to perform *in-vivo* tests of blood coagulation by non-equilibrium air plasma. The feasibility and effectiveness of this plasma spray to stop bleeding are studied.

This device consists of a pair of concentric electrodes, a ring-shaped permanent magnet, a blower, and a power supply. A photo of the device with the generated plasma spray is shown in Fig. 1. A nozzle was introduced to direct the flow of the plasma effluent as well as to cover the electrodes for safety, so that the high voltage (HV) central electrode would not be exposed. An air pump was used to provide the airflow, which has a flow rate of 3 ℓ/s and an average flow speed of 24.5 m/s at the nozzle exit.

Fig. 1: A photo of the portable plasma spray.

The emission spectrum of the plasma effluent outside the nozzle from 300 to 900 nm was scanned by a spectrometer. In this spectral range, the UV radiation from 300 nm to 400 nm was not detected and the intensive lines contributed by oxygen radicals appear only around 777.4 nm, which were emissions from the 5P state of atomic oxygen (OI) in the plasma effluent. The average temperature of the plasma effluent outside the nozzle was measured along the axis by a thermal probe. The peak value in the temperature distribution is less than 328 K (55 ^{0}C).

Tests were performed on four three-month-old pigs, weighing around 25 kg. Three types of wounds, straight cut and cross cut in the ham area, and a hole in an ear saphenous vein, were introduced to two of those [6]. The other two were introduced a hole in an artery of an ear. The wounds introduced on one pig were treated by the plasma; the other pig was used as an untreated control so the bleeding from each wound was stopped by itself. The time it took for bleeding to stop naturally without treatment was considered to be the natural clotting (bleeding) time. Each pig was first

injected with calmative-Stresnil and fastened on a table. The pig was then anesthetized with Isoflurance-Fluothane which kept it in a narcotized state.

Test ---Hole in an artery : In this test the emergency procedure by using a tourniquet to tie the ear, where an artery was being cut, was first introduced. An artery from a pig ear was identified and cut by a scalpel as shown in Fig. 2a. It caused a severe bleeding as shown in Fig. 2b. The plasma spray at a distance of 2.5 cm was then applied immediately to the cut as shown in Fig. 2c. An intermittent treatment approach, running the device with 2s on and 4s off, was adopted. After on-off 6 times with a total plasma exposure time of 12 s, the cut was clogged and bleeding was stopped (in less than 35s) as shown in Fig. 2d. On the other hand, without the plasma treatment the bleeding would last for more than 60s.

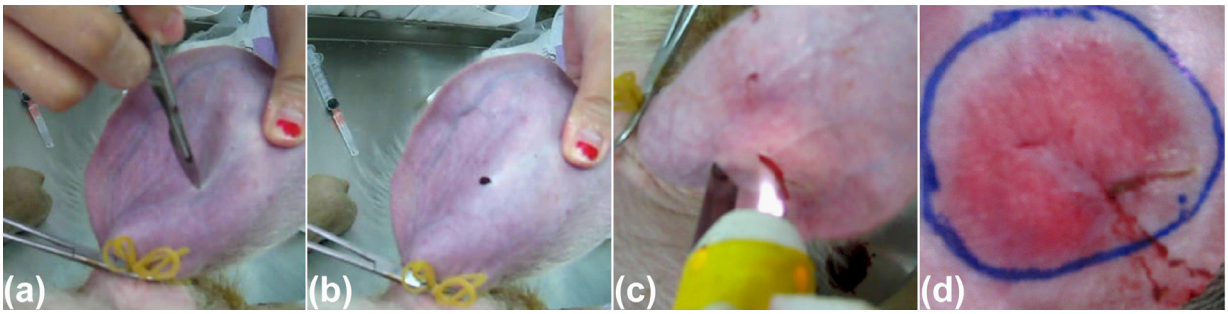

Fig. 2: (a) An artery in an ear of a pig is being cut, (b) bleeding from a cut artery, (c) plasma torch is applied to the cut, and (d) clogged cut after 35 s of intermittent plasma treatment.

The experimental results have shown that this plasma spray could rapidly stop bleeding. The atomic oxygen produced in the plasma effluent is likely the catalyst in the coagulation processes. When interacted with H_2O, atomic oxygen carried by the plasma effluent can produce large amount of reactive oxygen species (oxygen ions, free radicals, and peroxides). Studies have shown that platelets are a prime target for oxidants produced or released in the vascular lumen and, at the same time, they are also capable of endogenous generation of oxidants [7], [8]. It has also been shown that oxidants can affect several key steps of platelet function to enhance platelet aggregation [8]-[10].

It appears that the plasma treatment also produces a ring of pink color surrounding the cut as shown in Fig. 2d. It was found that this circular pink mark was independent of the wound and was not caused by a burn otherwise the inside area of the ring should suffer burn more severely. After about three days, this mark disappeared, and subsequently no other effect appeared in the exposed area in two-week observation. This suggested that this plasma spray would not cause any permanent effect on the exposed skin and tissues. This mark is likely attributed to the red blood cells accumulated underneath the marked skin. It suggests that the plasma effluent can penetrate the skin to block capillary blood flow into the exposed area. The blocked blood flow accumulates in the region surrounding the exposed area, showing a circular pink colored ring on the skin. Thus, the process of stopping bleeding by this plasma spray involves blocking capillary blood flow surrounding the wound as well as clotting the blood covering the wound. It explains why the low temperature non-equilibrium air plasma generated by the device can reduce the bleeding time drastically.

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Plasma effects on chronic infection models

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Résumé

Chronic infections, such as chronic ulcer and wound infections, lung and bronchoalveolar infections or infections of the urogenital tract represent the major concern for the modern therapy of infectious diseases. Causative agents of chronic infection are often resistant to the standard antimicrobial treatments. The resistance might be due to wide spreading of strains with multiple resistance to antibiotics, but as well due to changes in general metabolism that take place in bacteria during prolonged persistence in the human body. There are two major ways for bacteria extended survival, which are forming of biofilms and intracellular parasitism. Physical treatments present an alternative approach when effectiveness of chemical agents is weak due to natural pathogen or biofilm resistance. This study investigated the bactericidal effect of nonthermal plasma against bacteria in biofilms and inside eukaryotic cells.

The argon plasma source MicroPlaSter β (Tetsuji, 2008) was used that produced plasma by argon ionization with superhigh frequency electromagnetic field. To grow biofilms, Gram-negative bacteria *B. cenocepacia* and *P. aeruginosa* were used. A coverglass of 1.5 cm^2 in area was placed vertically into the flask where bacteria were cultured to allow a biofilm to form on its surface. The glass was removed after 72h, carefully washed with PBS and the biofilms were treated. Plasma or non-ionized argon treated *P. aeruginosa* biofilms were labeled with Live/Dead® cell viability kit (Invitrogen) providing a 2-color fluorescence assay of bacterial cells based of membrane integrity.

Gram-negative bacteria *Chlamydia trachomatis*, which are obligate intracellular pathogens were used as model intracellular microoganisms. *Chlamydia* were grown in the McCoy cells. A bactericidal effect of nonthermal plasma was studied at major stages of the chlamydial lifecycle including infectious extracellular elementary bodies (EBs) in water suspension; EBs attached to the cell surface during the process internalization into the cells; actively multiplying intracellular bacteria (reticulate bodies, RBs) that form in 24 h after infection; intracellular EBs formed in infected cells in 48 h after infection of the McCoy cells.

Biofilms rather than isolated bacterial cells represent a major form of bacterial persistence on the surface of both medical equipment and chronic wounds. In general, bacteria in biofilms are less sensitive to antimicrobial treatments (Davey, 2000). Dead bacteria prevailed in the plasma-treated biofilms while the argon-treated biofilms included more living than dead bacteria. Moreover, plasma-treated biofilms had higher concentrations of living bacteria at deeper layers compared to control, non-ionized gas treated biofilms. Presence of dead in bacteria in argon-treated biofilms was not related to an effect of non-ionized argon gas. Quantitative assessment of biofilm treatment reveal the bactericidal effect with only 0.005% to 2% of survivals.

Both extracellular and intracellular *Chlamydia* forms were highly sensitive to plasma treatment. About 0.01 % bacteria survived treatment. The noticeable effect of plasma treatments on intracellular bacteria raised the question concerning susceptibility of host eukaryotic cells to argon plasma. In 24 h after 2 minutes treatment, about 35 % drop in the amount of viable epithelial McCoy cells was observed in regarding to control cells. There was no difference in viability between infected and non-infected cells.

Thus, plasma might be effective in elimination of pathogenic bacteria in biofilms and within cells, which are main bacterial localizations during chronic infection process.

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Physical mechanisms of plasma assisted wound healing: Production and delivery of active species

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Résumé

The physical mechanisms of plasma assisted wound healing are discussed. Experimental data on plasma production of reactive species and depth of penetration into tissue (in vitro and ex vivo) are presented.

Introduction

In recent years, the range of atmospheric pressure applications for medical and biological purposes is growing fast, and a new field of Plasma Medicine was formed [1-2]. Various types of plasmas, both thermal and non-thermal, are now widely studied for the purposes of blood coagulation, sterilization of living tissues, treatment of various wounds and burns, gastroenterological diseases, and even cancer. This opens up new horizons in both medical and physical sciences, as well as in biomedical and electrical engineering.

Cold atmospheric pressure plasma discharges have been shown to be effective when applied for sterilization and decontamination purposes, wound healing, blood coagulation, and other relevant medical and biological applications. Action of specific charged or neutral active species or radiation is frequently associated with the corresponding specific effect (e.g., anti-inflammatory effect of nitric oxide (NO), and highly oxidative hydroxyl radical and other reactive oxygen species (ROS)). Here we report the results on measurement of simultaneous production of anti-oxidant NO together with oxidative ROS in liquid media and their delivery into agarose gel and tissues by microsecond atmospheric pressure spark discharge.

Materials and methods

In our study we have used DC spark discharge plasma in a pin-to-hole electrode configuration (PHD plasma - Figure 1), previously described in [3] and [4]. The discharge was ignited by applying high positive potential with magnitude of about 4 kV to the central electrode. This resulted in a formation of dense energetic discharge which exists for about 35 μs with average energy of about 1.8 J per pulse.

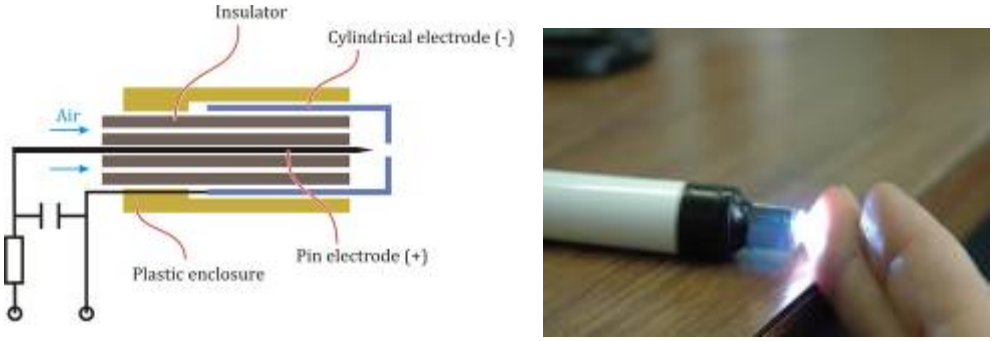

Fig. 1: General schematic of the Pin-to-Hole spark Discharge (PHD) plasma system and a photograph of the discharge in operation.

Measurements of hydrogen peroxide (H_2O_2), and nitric oxide (NO) produced by plasma in phosphate buffered saline (PBS) were done using fluorescent dyes, Amplex UltraRed reagent (Invitrogen), DAF-2 (Cayman Chemical) respectively according to manufacturers' protocols. Superoxide (O_2^-) was measured indirectly by adding superoxide dismutase (Fisher Scientific) into PBS solution containing Amplex UltraRed reagent before the plasma treatment. Fluorescence was measured using an LS55 (Perkin Elmer) fluorescent spectrometer equipped with well plate reader accessory.

Measurements of H_2O_2 and pH penetration into agarose gels (0.6%, 1.5%, 5% of agar) and tissues were done using the same fluorescent dyes. In the case of H_2O_2, the dye was placed in between 1 mm thick agar

slices and incubated for about 15 minutes before the treatment in order to provide presence of the dye in the agar volume; for the pH measurement, the agar gel was prepared by adding fluorescein (Sigma) dye before its solidifying. In order to measure the H_2O_2 and pH in tissue, the dyes were inject using a syringe into a skinless chicken breast tissue to the depth of about 1-2 mm.

Results

On Figure 2 the results of plasma production of H_2O_2 and NO in PBS solution are shown. The results of H_2O_2 and pH measurements in agarose gels and tissues are shown on Figure 3.

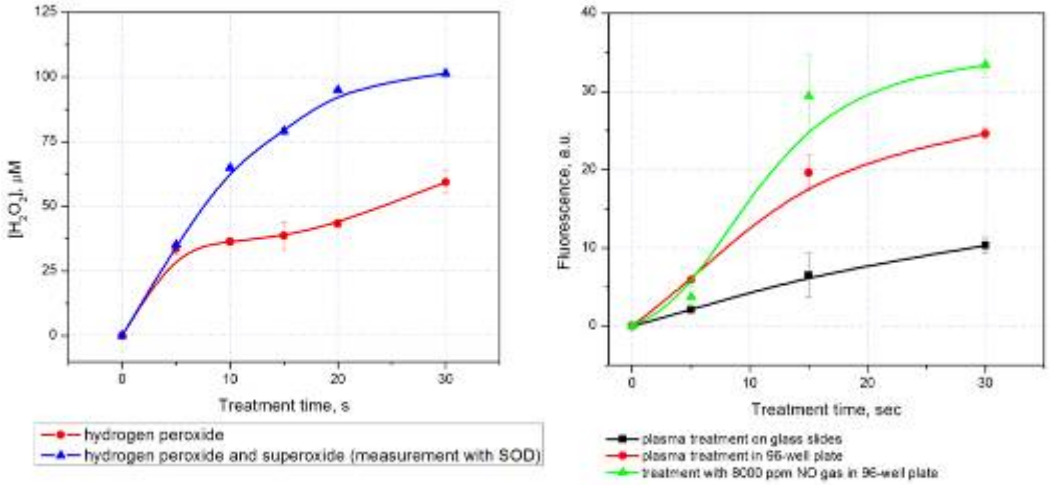

Fig. 2: Pin-to-Hole spark Discharge (PHD) production of H_2O_2 (left) and NO (right) in PBS.

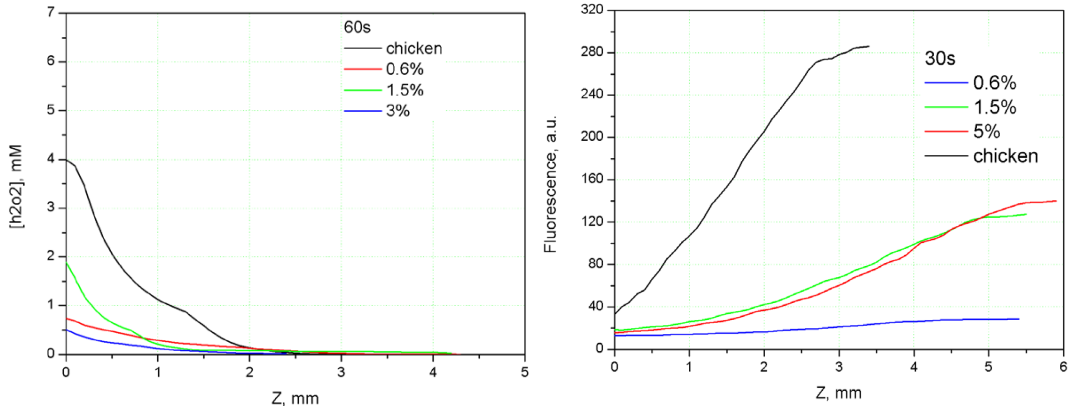

Fig. 3: The profiles of H_2O_2 (left) and pH (right) in agarose gels and tissue after the plasma treatment.

The results show that PHD discharge effectively produces both ROS and RNS species in the treated media, and these species may be delivered into the tissues to the depths of several mm, therefore providing not only surface effects (inactivation of pathogens, first of all), but also therapeutic effects inside of treated tissues.

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Generator of focused shock waves in water for biomedical applications

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Résumé

Generator of focused two successive (tandem) shock waves in water (FTSW) based on the production of two pressure waves generated by underwater multichannel electrical discharges has been developed. In this work principles of generation of FTSW in water and the results on biological effects of focused tandem shock waves will be presented on *in vitro* and *in vivo* experiments.

Introduction

The generation of focused shock waves by a high-current spark discharge in water has been studied for many years in connection with extracorporeal lithotripsy of kidney stones. Early experiments showed that the rarefaction part of the generated shock waves is weak at the focus [1]. This weak rarefaction wave is due to the fact that the high-current spark discharge generates in water a spherical shock wave. Because the shock wave is situated in the focus of a metallic semi-ellipsoidal reflector, through the secondary focus is propagated without a change in the waveform. Cavitation bubbles created by the rarefaction wave and by the collapse of the discharge bubble relax near the discharge channel, and thus, the rarefaction wave is strongly dampened at the secondary focus. The great success of lithotripsy has stimulated research on the application of focused shock waves in other branches of medicine. Most of this attention has been focused on the possible treatments of some types of cancers. However, unlike kidney stones, cancer tissues are acoustically the same as the surrounding healthy tissues, and shock waves of the type used in lithotripters barely interact with cancer tissues. To achieve localized action of shock waves in an acoustically homogeneous medium such as soft tissue, the utilization of two subsequent (tandem) shock waves seems to be a possible option assuming that the first shock creates at the focal region an acoustic non-homogeneity (rarefaction wave) in water on which the second shock will dissipate.

For this purpose we have recently developed a generator of focused two successive (tandem) shock waves in water (FTSW), which is based on the production of two cylindrical pressure waves generated by underwater multichannel electrical discharges at two porous ceramic-coated cylindrical metallic electrodes of different diameter [2,3]. The primary cylindrical pressure waves generated at each electrode are generated with a set time delay between them, focused by a metallic parabolic reflector to a common focal point and only close to focus are transformed into a strong shock wave. We have found that at time interval of 10-15 μs between the two shocks the second, originally pressure wave, is strongly attenuated at the focal region and reaches the focus as a rarefaction wave. Amplitude of the pressure wave is up to 100 MPa, while the amplitude of the rarefaction wave falls down up to -80 MPa, producing thus at the focus a large number of cavitation bubbles. The collapsing cavitation bubbles produce secondary, short-wavelength shock waves and fast microjets that may be able to interact with cell-scale structures. In this work principles of generation of FTSW in water and the results on biological effects of focused tandem shock waves will be presented.

Experimental

Fig. 1 shows scheme of generator of focused two successive (tandem) shock waves in water. The generator is divided by acoustically transparent membrane into two parts. The first part, which is filled with highly conducting saline solution (with the conductivity of the order of tens of mS/cm), consists of two metallic cylindrical high-voltage electrodes of different diameter covered by a thin porous ceramic layer (composite anode) placed along the axis of the outer metallic parabolic reflector (cathode). The pulsed high voltage applied to the composite electrodes is provided by a pulse power supply that consists of two 0.4 μF capacitors charged up to 30 kV and two triggered spark gaps (SG). The focal point of the

reflector is situated in the second part of the generator, which is filled with a tap water. Design of the composite electrode allows simultaneous generation of a large number of filamentary discharge channels in water, which are distributed almost homogeneously along the whole surface of the electrode at a moderated applied voltage (20-30 kV) [4]. Every discharge channel creates a semi-spherical pressure wave, and by superposition of all of the waves a cylindrical pressure wave propagating from the anode is formed. The primary cylindrical pressure wave is focused by a reflector and near the focus it is transformed into a strong shock wave.

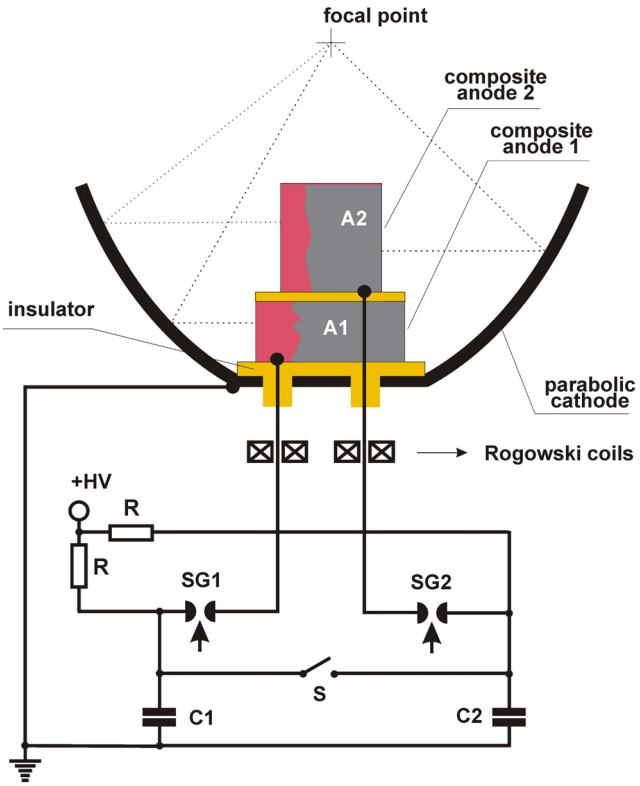

Fig. 1: Scheme of FTSW generator.

Results

Presented FTSW generator is used for investigation of biological effects of focused tandem shock waves. We have recently demonstrated *in vivo* using laboratory rabbits that such tandem shocks are capable of causing localized lesions at a predictable location deep inside soft animal tissue. In subsequent *in vitro* experiments, we have demonstrated that exposure of carcinomas cells to the tandem shocks results in cells membrane perforation and, at a higher dose, damage to the cells. Because healthy and cancerous tissues do not differ acoustically, the localized action of the shock waves in such an acoustically homogeneous medium (soft tissue) is generally due to cavitation bubbles produced by the rarefaction wave. Local (micrometer dimensions) thermal effects accompanying the collapse of cavitation bubbles (sonoluminescence) and production of chemical radicals may also play roles in cell damage. *Ex vivo* experiments on laboratory mice revealed that the tumors from the exposed cells grow much slowly than in controls. Currently, *in vivo* experiments on synergistic effects of the tandem shocks in combination with cytostatic and photo-/sono-sensitive anticancer drugs on the growth rate of tumors are preformed on laboratory rats. Preliminary results of these experiments and prospects of FTSW to enhance cytotoxic efficiency of anticancer drugs will be discussed.

Acknowledgement

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Characterization of an intermittent negative dc - corona discharge in argon designed for medical applications

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Résumé

Beside the characterization of biological and medical effects of plasmas a profound physical understanding of such discharges is required. Consequently, plasma sources under investigation in plasma medicine and biology had been developed or adapted for the treatment of biological samples up to living objects. Such plasmas have to be investigated under realistic conditions, which is a challenging task for diagnostics as well as simulation. In this contribution the investigation and characterization of a novel plasma source developed for special medical applications by fast optical and spectroscopic methods is described.

Introduction

Plasma medical applications require non-thermal plasma sources for atmospheric pressure operation. The design and properties of such plasma sources is determined by the application itself [1]. In particular for the treatment of human skin the gas temperature of the plasma should be lower than 300K, which can be achieved by pulsed operation in the nanosecond time regime. Recently a novel device for the generation of a nanosecond pulsed cold plasma using a dc-power supply was described [2]. This so-called hairline plasma source produces a very thin plasma filament. Due to its geometrical parameters it is particularly suitable for the treatment of microscopic cavities, which might help to overcome problems in endodontic treatment in dentistry (fig. 1).

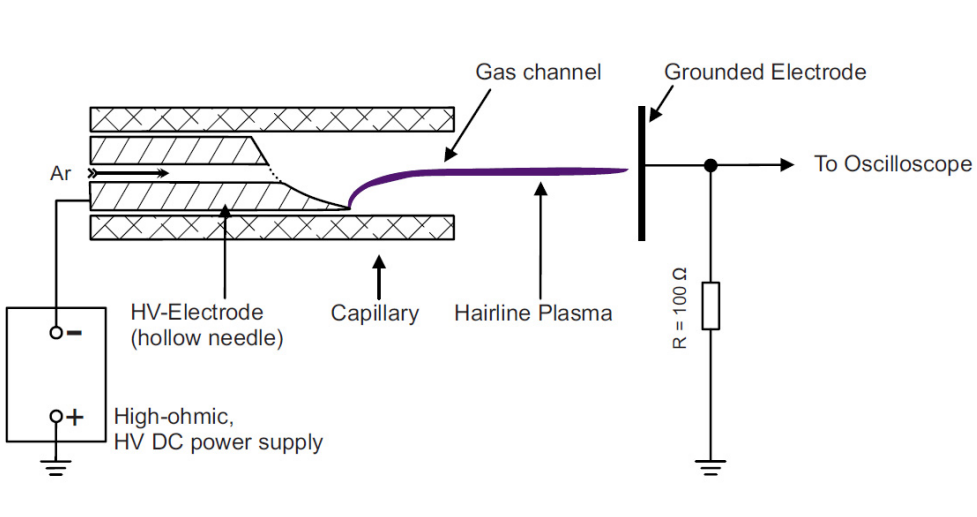

Fig. 1: Schematic of the hairline plasma device (left) and in operation on a prepared human tooth showing the plasma filament in the root canal (right) [2]

The anti-microbial efficiency has been demonstrated by exposing Escherichia coli colonized agar plates to the discharge. The path where the plasma filament was moved over the agar shows a localized antibiological effect of the plasma. Current pulses were measured and its repetition frequency was derived. Pulses between 0.4 and 1.4 A with FWHM of about 10 ns and repetition frequencies from 1 to 3 kHz were obtained Furthermore a broad characterization of the plasma filament by optical emission spectroscopy in the VUV and in UV/VIS spectral range was performed. In this contribution we will focus on the discharge development and its influence by operation parameters.

Experimental

The intermitted dc-corona plasma device (see fig.1, left) consists of an inner high voltage hollow needle electrode (outer diameter 0.8 mm) connected with the negative pole of a high ohmic high voltage dc - power supply (U = 1 - 14 kV). The needle electrode is surrounded by an insulating capillary forming a gas channel in front of the electrode (length of some millimetres). The feed gas used here is argon which is supplied through the inner channel of the hollow needle. The second electrode is connected with the grounded positive pole of the power supply. The distance between the end of the capillary and the second electrode can be up to 1.5 cm. In dependence on the electrode distance and the applied voltage a plasma filament appears between both electrodes with a typical radial extension of 30 µm and a maximal length of 1.8 cm. In order to investigate the discharge development an ICCD camera equipped with a far field microscope was used. The spatially and temporally resolved development of light emissions at selected wavelengths was performed by means of Cross-Correlation Spectroscopy [3].

Results and discussion

The fig. 2 shows an example of the discharge development derived from the measurements at λ= 337 nm and 391 nm, which are the 0-0-transitions of the molecular band of the second positive system of N_2 and the first negative system of N_2^+, respectively. The argon plasma is operated at open atmosphere causing the mixture with the surrounding air. In the figures the axial coordinate is the ordinate, while the abscissa is the time scale (which is only relative due to the nature of CCS measurements). The tip of the needle electrode (cathode) is at 0 mm, while the horizontal line at 5 mm shows the end position of the quartz tube. The grounded electrode (anode) is located at 8.2 mm. The number of counted photons is colour coded in logarithmic scale.

The results demonstrate the highly complex development of the plasma filament. Five subsequent phases are investigated, namely (i) pre-phase (t < 125 ns) with maximal emission in front of the capillary, (ii) anode directed front of light (negative streamer) (t= 125 … 140 ns), (iii) cathode directed front or streamer (t= 140 … 155 ns), (iv) plasma column with high energetic species (t= 145 … 160 ns) and (v) decay phase (t> 160 ns). The contribution will discuss the discharge formation in detail with special attention to biomedical applications.

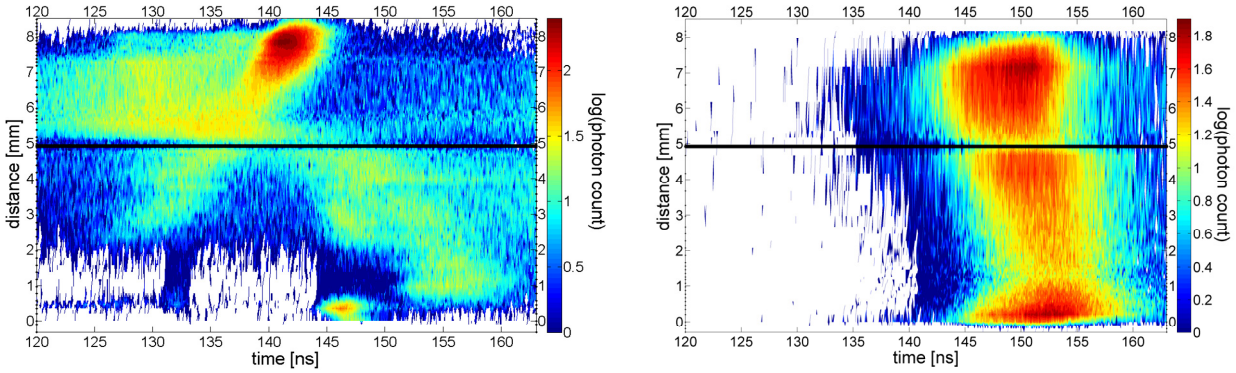

Fig. 2: Spatio-temporal development of intermittent negative dc-corona filaments in flowing argon for N_2* (left; λ= 337 nm, 11 eV excitation energy) and N_2^+* (right; λ= 391 nm, 19 eV exc. energy)

Acknowledgements

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Low temperature atmospheric argon plasma: Diagnostics and medical applications

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Résumé

We present here the methods of optical and probe diagnostics of low temperature plasma produced by microwave generator with frequency of 2.45 GHz at low power (~ 150 W) and at low temperatures of a gas (argon) flow (< 40°C). The spatial distribution of a brightness temperature in plasma was obtained. The profile of gas temperature near the torch outlet was measured. High-resolution vibrational-rotational spectrum in a wide range of wavelengths was derived by the method of optical emission spectroscopy. Probe measurements of the floating potential of plasma were carried out. The estimation and adaptation of parameters of a plasma flow (temperature, velocity, ion number density) according to medico-technical requirements were obtained.

Introduction

Use of plasma in medicine was connected with its thermal effect on a processed surface until nowadays. However, non-isothermal plasma influence has been of great interest recently because of a possibility of its application for obtaining of various effects: sterilizations, healing of wounds, cell detachment, etc. Presented are the methods of optical and probe diagnostics of low temperature plasma produced by microwave generator with frequency of 2.45 GHz at low power (~ 150 W) and at low temperatures of a gas (argon) flow (< 40ºC).

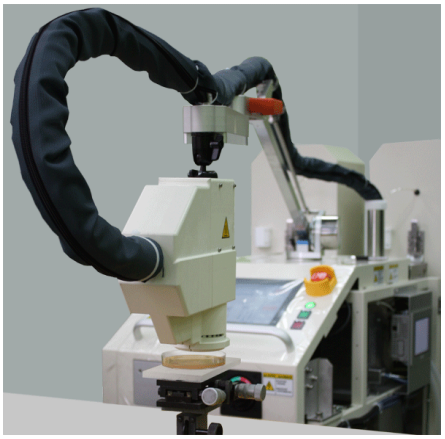

Fig. 1: The generator of low temperature argon plasma

Diagnostics and experiments

The generator of low temperature argon plasma (fig. 1) is the electrode of complicated configuration placed in the grounded metal cylinder with connecting pipe for delivery of buffer gas of argon. When pumping argon through a discharge gap with speed of 4-8 l/min and providing magnetron power 120-150W a flow of microwave discharge plasma is obtained and small plasma channels between the end of the electrode and an internal surface of the grounded cylinder are formed. Diameter of a plasma stream in such conditions is not less than 30 mm. Inert gas consumption controls is carried out by system of monitored inflow. Speed of argon flow defines a length of plasma torch which was about 35-45 mm in our experiments. To derive such parameters of a plasma torch as temperatures, floating potential, plasma components, we chose typical parameters of biological experiments with microorganisms, mammal tissue and person (power of the discharge - 120W, speed of argon flow - 5 l/min, orifice gas - argon with purity of 99.998 %). The plasma torch was fastened on an optical table with possibility of its exact positioning in space by means of micrometric screws (fig. 2). The room temperature was 19ºC. Optical diagnostics was used for obtaining of spatial distribution of a brightness temperature in plasma torch behind a discharge outlet (fig. 3). Survey of plasma was made by a digital

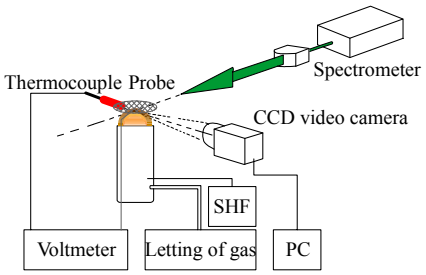

Fig. 2: The generator of low temperature argon plasma

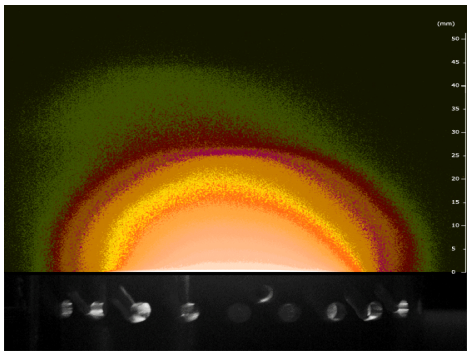

Fig. 3: The distribution of a brightness temperature in plasma torch behind a discharge outlet

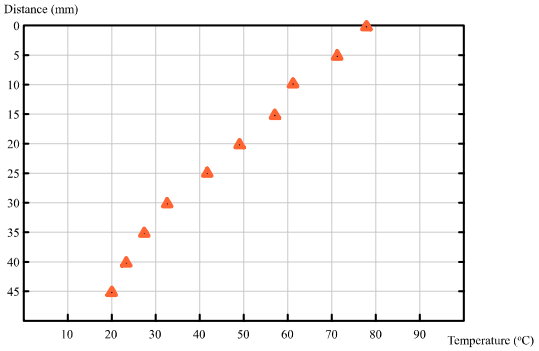

Fig. 4: Gas temperature distribution in plasma behind a torch outlet

CCD-camera. We optimized the location of the camera during the experiment, its time resolution varied in a range of 10-40 μs. The image of plasma of a torch received by specified way was processed then on the computer by means of specially developed software. We measured gas temperature in plasma behind a torch outlet by the shielded thermocouple to exclude distortions of measurement results owing to plasma influence on a thermocouple current (fig. 4). We used chromel-alumel type of the thermocouple that is suitable for temperature measurements in a range up to 1100°C.

Spectrometer Avesta ASP 150TF was used for realization of a method of optical emission spectroscopy of a torch to obtain high-resolution vibrational-rotational spectrum in a wide range of wave lengths. The width of the spectrometer entrance slit was 100 μs. The information about emission was gathered by series of CCD-detectors connected with the computer. Monochromator resolution with 2400 dashes of a lattice per 1 mm was 0.016 nm. Exposition time of CCD was generally 0.02 s, signal was amassed during 10 expositions. The area of a covering of a spectrometer was 185-1105 nm. Plasma emission was focused on the end of the optical fiber that was connected with spectrometer.

We designed special, so-called net probe to measure a floating potential of plasma. With the help of it we obtained longitudinal profiles of a plasma field along the torch. The net probe was kept by a molybdenum holder and covered a plasma torch completely. When measuring we regulated a position of a torch to a net probe from 0 to 45 mm. the profile of a floating potential of a grid in plasma torch along an axis z is presented on fig. 5. Plasma density in this area is estimated in a range of 10^5-10^6 cm^{-3} that is sufficient for an effective charging of small biological objects. In this case charging time is defined by the 100-th and tenth fractions of a second that is much less than time of processing of working surfaces. Further, measurements had shown that in an interval of 5-35 mm behind torch outlet the potential of an electrode decreased to 5V.

During biomedical experiments we investigated an influence of low temperature argon plasma on microbal biofilms and on animal (rats) while modeling an infection. Quantitative estimations of a survival of bacterial cells in vitro after an irradiation were derived. The obtained results let us assert that the considerable percent of bacteria in a biofilm perishes after plasma effect. We also estimated an efficiency of healing of a wound by reduction of the area of its surface.

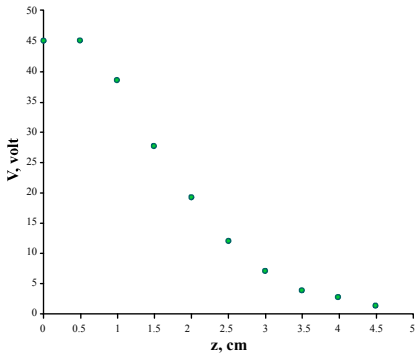

Fig. 5: Profile of a floating potential of a grid in plasma torch along an axis z.

Conclusion

The estimation and adaptation of parameters of a plasma flow (temperature, velocity, ion number density) according to medico-technical requirements were obtained. Research results had shown an efficiency of low temperature argon plasma effect on biological objects in vitro and in vivo for disinfecting and healing of the festering wounds.

Acknowledgments

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Experimental study and sterilizing application of non-thermal plasma technology

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Résumé

Non-thermal dielectric barrier discharge has been constructed that can be operated at atmospheric pressure consists of two parallel electrodes, one of which covered by a dielectric material. An ultra-short pulsed dielectric barrier discharge is employed to inactivate microorganisms in contaminated water. Experimental results indicate that after 180 s pulsed plasma discharges killing the targeted microorganisms in the water sample that contains some pathogenic microorganisms including Heterotrophic Plate Count (HPC) more greater than 210 CFU/ml and coliform bacilli greater than +240/100ml. The water sample was collected from River Nile in Cairo, Egypt.

Dielectric barrier discharges DBD or silent discharge plasmas are such that one or both of the electrodes are covered with dielectric layers. In conventional atmospheric pressure discharges, arcing results in localized heating and non-uniform processing of the gas. In DBD, however, the dielectric surfaces serve the role of a capacitor in series with the plasma. The plasma in DBD consists of a "forest" of micro-streamers.

Fig. 1 shows the schematic diagram of the pulsed power generator used in this paper. The generator is consisting of a negative dc source, a Blumlein-type pulse-forming network (E-PFN), and a dynamic spark gap switch. A triggered spark gap switch was used as a closing switch of E-PFN. E-PFN had 4 stages of LC ladder, which were composed of 5 nF of capacitor and 3 μH of inductor. The characteristic impedance ($2\sqrt{L/C}$) and the pulse width ($2N\sqrt{LC}$) of E-PFN, calculated from capacitance (C) and inductance (L) of the LC ladder, and number (N) of LC ladder stages were approximately 49Ω and 1.0 μs, respectively.

A charging resistance value of 50kΩ is chosen in the present case which corresponds to a charging RC time constant of 1 ms, which is 40 times faster compared to the repetition rate of the pulse.

The charging voltage into E-PFN was varied between 10 kV up to 30 kV. The applied voltage to and the discharge current through the discharge chamber were measured using a voltage divider (Home made), which was connected between the two electrodes, and a current monitor, which can be located upon returning to the ground. The signals from the voltage divider and the current monitor were recorded in a digitizing oscilloscope (Lecroy, USA) with a 200-MHz bandwidth.

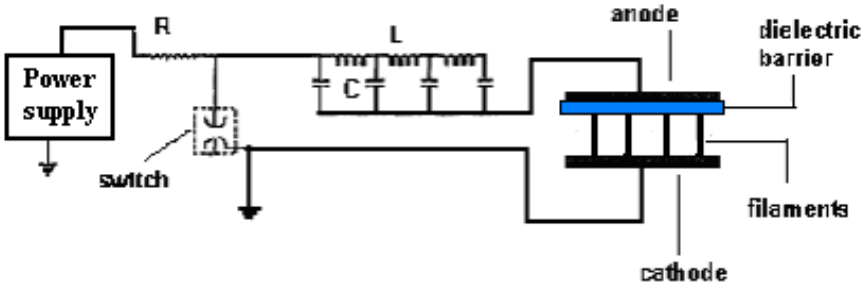

Figure1. Schematic diagram of the pulsed power generator.

Fig. 2 shows the configuration of the two plane parallel electrodes at least one which is covered by dielectric of thickness a few millimeters.

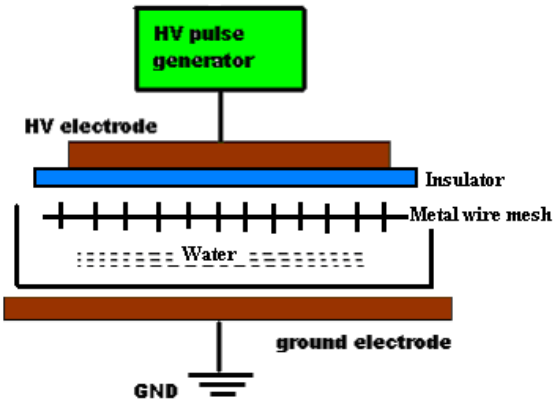

Fig. 2: Geometry of DBD discharge

Fig. 3 shows an exemplary of the waveforms of the applied voltage to and discharge current through the discharge chamber with 10-kV (a), these measurements allowed the determination of the power (b). The width of filaments lies between 15 – 20 ns and the period of filaments about 30 ns.

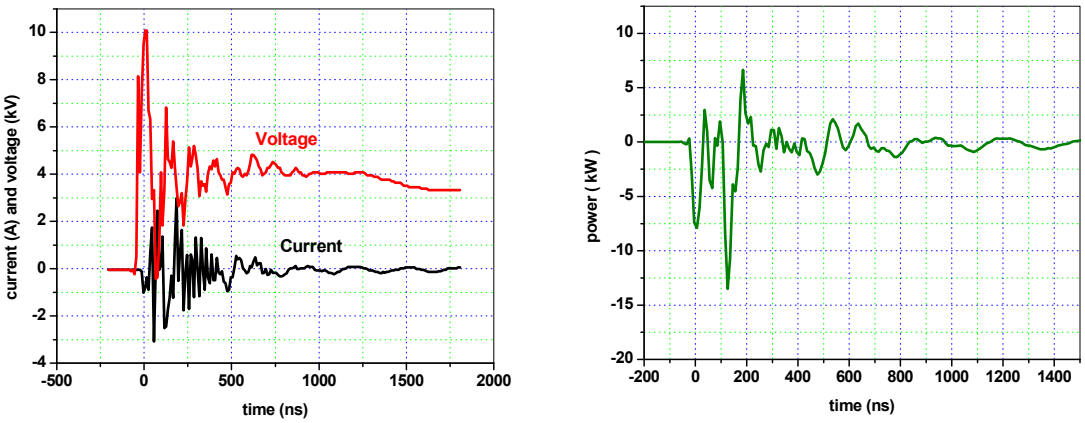

Fig. 3: The current, the voltage (a) and power waveform (b) of the DBD device

By using a gas flow the discharge is capable of changing into a homogeneous pattern. Figure 4 indicate that the filamentary discharge with air at atmospheric pressure and the homogeneous discharge using argon gas with flow rate 60 lpm, the discharge with more filaments.

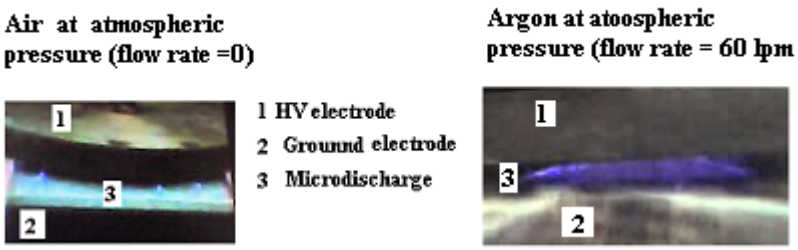

Fig. 4: Photograph of micro-discharges pattern in a 2 mm air gap (a) the filamentary discharge (b) the homogeneous looking discharge.

Organic compound destruction in dynamic plasma–liquid systems

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Résumé

The processes of organic compound (phenol and cation-active surfactants) destruction and inactivation of cultures (*Escherichia coli*) in water solutions, which occurs under the influence of plasma, and growth of cultures (spore culture of *Bacillus cereus* B4368 and the Gram-negative culture *Pseudomonas fluorescens* B5040) in water after plasma treatment was investigated to different dynamic plasma-liquid systems (PLS). The breakdown products of phenol and cation-active surfactants detected with absorption spectroscopy. The bacteria destruction was analysed with application of ultraviolet absorption and luminescence spectroscopy. It was shown that the sterilisation of water with *Escherichia coli* under influence of plasma secondary discharge with "liquid" electrode at atmospheric pressure may amount to 100%. Gram-negative culture more intensively cultivates in water after low-pressure plasma treatment, spore culture - after plasma treatment of atmospheric pressure. At plasma sterilisation the bacteriostatic effect is observe. The most effective system for phenol plasmolytic destruction in water solutions are the secondary discharge with a liquid electrode at atmospheric pressure and PLS, based on the impulse discharge in the gas channel with liquid wall.

Introduction

The problem of complete cleaning for the industrial wastewaters from organic high active and toxic substances (HATS) is important enough and simultaneously difficult decided. However this problem can not be considered decided. Apparently, radiochemical and plasmachemical technologies are represented by most perspective, as allow to achieve the greatest speed destruction of substances at the expense of high-energy concentration. However, it is necessary to take into account, that toxic substances are, frequently, the complex high-molecular compounds. Therefore destruction of HATS results in occurrence not only products of disintegration, but also wide spectrum more complex high molecular of compounds [1]. The chemical reactions both in radiochemical and in plasmachemical systems can proceed with participation of the electronic-exited particles, which practically are not investigated today. It is specified that by high probability of occurrence unknown ealier substances at the data technologies. Therefore now the transition to complex technologies on a basis of plasmachemical processes begin. The opportunities of plasma-bio technology were considered at water clearing from chlorophenols in work [1] and were shown, that the transition to complex technology of water clearing results to synergism.

However, the destruction of toxic components by microorganisms can result in occurrence the mutants. It means that after biochemical destruction of high active products of preliminary plasma water clearing from initial toxic pollution it is necessary to provide inactivation of microorganisms in water. This work is devoted to development of similar multistage technology on base of plasma-bio-plasmachemical processes. The base scheme of the proposed technology includes the plasma module for the preliminary treatment of initial wastewater, the biochemical modules (biodestruction, biosorption and biosedimentation) and the modules for plasma inactivation of microorganisms in water.

2. Experimental technique

The process of organic compound destruction in water solutions, which occurs under the influence of plasma, was investigated to different plasma-liquid systems (PLS) in this work. The organic solutions in distillated water was treated by plasma of secondary discharge with a liquid electrode at low pressure [2] of secondary discharge stimulated by transverse arc at atmospheric pressure [3] of DC discharge in the gas channel with liquid wall and the additional excitation of ultrasonic field in liquid [4, 5], Pulse discharge in gas channel with liquid wall [4] and the discharge in reverse-vortex gas flow of "tornado" type [6].

2.1. The surfactant and phenol destruction in water solutions by plasma treatment. The plasma influence on the molecular structure of the surfactants in water solution was studied. The alcylpiridinium salts (pentadecyl piridinium bromide, cetyl piridinium bromide, tetradecyl piridinium bromide) and quaternary ammonium salts (tetradecyl three ethyl ammonium bromide, cetyl threemethyl ammonium bromide) have used as cationic surfactant models in the present work. The reference solutions with 10-3 M concentration were prepared by distillate water solution of the purity crystal matters. Other solutions were prepared by water diluting. The sensitive and selective sorption-photometric method have been using for surfactant detection in solutions after plasma treatment. This method permits to determine less than 1 μg/l cationic surfactants for all types of it. Phenol destruction in water solutions was investigated in a range of concentration 10-6 - 5 10-1 M with use the spectrophotometer analysis in UF area of a spectrum (200 - 500 nm).

2.2. Plasma inactivation of microorganisms. *Escherichia coli* was used as test culture at study of decontamination action of plasma processing. The plasma processing was carried out in the experimental module with the secondary discharge with a liquid electrode at low pressure ~ 10 torr [2] and in the plasmachemical reactor on the base of secondary discharge with the "liquid" electrode at atmospheric pressure [3].

2.3. Growth of cultures in water after plasma treatment. The various secondary discharges of low and high pressure with a liquid electrode used at plasma treatment. The spore culture of *Bacillus cereus* B4368 and the Gram-negative culture *Pseudomonas fluorescens* B5040 were used as test cultures. Cultures cultivated within 18 hours on nutritious environment no. 284 containing 5 g/l gluconate. Then these cultures accommodated in distillate water past plasma processing and investigated intensity of their growth.

3. Basic results

The results of work indicate the efficiency of plasma destruction of phenol and surfactants, inactivation of micro-organisms at plasma of water and allow to make a choice of cultures for designing of biodestructeres of products of preliminary plasma treatment in complex plasma-bio-plasmachemical technology of waste water treatment. The most effective system for phenol plasmolytic destruction in water solutions are the secondary discharge with a liquid electrode at atmospheric pressure and PLS, based on the pulse discharge in the gas channel with liquid wall. The complete inactivation of *Escherichia coli* can occur at low enough power inputs ~ 6 kW hour $м^{-3}$ by the plasma inactivation with use of the secondary discharge at low pressure with a liquid electrode in optimum modes: at negative polarity of a liquid electrode. The degassing of a solution as a result of an exposition it at the lowered pressure (10-30 torr), the burning of the auxiliary independent discharge above a surface of a solution did not influence on vital functions of *Escherichia coli* culture. The essential influence of thickness of a solution above an electrode in a solution was not noticed also on vital functions of *Escherichia coli* culture. The water treatment by plasma of secondary discharge with “liquid” electrode at atmospheric pressure can be reduced to 100 % of water with *Escherichia coli* sterilization also. The plasma sterilization of water at atmospheric pressure is accompanied by bacteriostatic effect. Appearance of luminescence in a water solution is observed after completely sterilization. Gram-negative culture more intensively cultivate in water after low-pressure plasma treatment, spore culture - after plasma treatment of atmospheric pressure.

Acknowledgement

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Bacterial biofilm inactivation by gas discharge plasma: overview and future perspectives

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Résumé

Bacterial biofilms are more resilient to standard killing methods than free-living bacteria. Our results show that gas discharge plasmas are a novel alternative to inactivate/sterilze biofilms. However, viability experiments have to be carried out before drawing the conclusion that plasma kills cells based solely on their culturability. Non culturable cells retain viability and pathogenicity after short exposures to plasma.

Overview

Most studies dealing with growth and physiology of bacteria have been carried out using free living (planktonic) cells. These studies have provided extensive information regarding the basic molecular mechanisms controlling the growth of individual bacteria. However, most bacteria live primarily in communities referred to as biofilms. Biofilms are microbial communities embedded in an exopolysaccharidic matrix and responsible for undesirable effects including disease, biofouling, pipe plugging, corrosion, dental plaque, and prosthetic device contamination, just to mention a few. Cooperative interactions among members of the biofilm make conventional methods of controlling microbial growth often ineffective. Therefore, there is a need to develop novel inactivation/sterilization tools and the use of gas discharge plasmas represents an alternative to traditional methods (revised in [6]).

My laboratory first studied biofilms produced by *Chromobacterium violaceum*, a bacterium present in soil and water. Biofilms were subjected to plasma for different exposure times and 99.6% of culturable cells were inactivated after a 5-minute treatment [1-5]. Atomic force microscopy (AFM) images revealed sequential changes in cell morphology occurring during plasma treatment [4]. However, physiological and metabolic determinations, and fluorescence microscopy showed that non-culturable cells were still alive after short plasma exposure times. These results indicated that viability experiments are indispensable before drawing the conclusion that plasma kills cells based solely on culturability [5].

We are presently studying plasma-mediated inactivation of *Pseudomonas aeruginosa* biofilms. This bacterium is an opportunistic pathogen that preys on victims with compromised immune systems, patients on respirators, and causes infections of burned tissue and colonization of catheters and medical devices. We have recently reported the effect of plasma on *P. aeruginosa* strain PAO1 biofilms grown on borosilicate coupons [7]. We are now looking at the effects of gas discharge plasma on 1, 3, and 7-day-old biofilms of *P. aeruginosa* grown on polycarbonate, stainless steel, or borosilicate coupons in a CDC biofilm reactor (BioSurface Tech, MT). An atmospheric pressure plasma jet is generated with an Atomflo^{TM} 300 reactor (Sur*fx* Tech. CA) using a mixture of He and N_2 gases. Biofilms are exposed to plasma for various exposure times and processed to determine CFUs/mL after incubation. Results indicate nearly 100% of biofilm inactivation after 5-minutes of plasma exposure. The inactivation kinetics are similar for 1, 3, and 7 day-old biofilms and show a rapid decline in the number of surviving cells followed by a much slower decline. No differences were observed for the three materials used. The inactivation kinetics is similar to the one obtained for *C. violaceum* biofilms [5], suggesting that the method is useful regardless the type of biofilm treated. AFM images show changes in cell morphology and biofilm structure for various plasma exposure times. Micromechanical properties of biofilms are studied through force versus distance curves [7].

One of the issues of more concern regarding work dealing with plasma-assisted cell inactivation is that, in most of the cases, the lethality of plasma is assessed based on the number of cells that can be cultured after the treatment. However, bacterial cells can respond to stress by entering a viable-but-non-culturable (VBNC) state. The fact of not being able to culture bacteria after plasma treatment cannot be considered as a clear-cut indication that the cells are dead. The VBNC state is a survival mechanism of bacteria facing environmental stress conditions. Bacteria enter into this dormant state in response to one or more

environmental stresses, which might otherwise be ultimately lethal to the cell. When cells are VBNC they are unable to produce colonies in an agarized medium but they are still alive and may retain pathogenicity. To test this hypothesis for *P. aeruginosa* biofilms, we developed a virulence assay using lettuce plants. The central vein of healthy lettuce leaves was injected with a bacterial suspension exposed to plasma for different exposure times. A non-exposed control was also included. Results show that *P. aeruginosa* cells that are non culturable after a short exposure to plasma, are still pathogenic.

Our results clearly show that bacterial biofilms can be inactivated by using gas discharge plasma. The architecture and the stability of the biofilm, together with cell culturabilty, are impacted by the plasma treatment. These results are evidence of the potential for plasma as an alternative sterilization method against biofilms. However, viability experiments should always be carried out before drawing the conclusion that plasma is useful to kill cells based solely on measurement of culturable cells. Research is being carried out in our laboratories to try to better understand the mechanism leading to cell inactivation. We expect that our study will provide the fundamental understanding of plasma-assisted biofilm inactivation and its mechanisms and build the basis for the future development of the technology.

Future perspectives

The results discussed are an evidence of the potential of gas discharge plasma to inactivate bacterial biofilms. The technology is clean and reported to be safe in medical settings for both the patient and the operator, although more research is needed to test the safety of the procedure. In any case, care has to be taken before drawing conclusions about the complete removal of biofilm-forming cells. A single cell detached from a biofilm is able to adhere to a surface and trigger the development of a new biofilm. Therefore, if the technology is to be applied to pathogenic organisms in health-related settings, this aspect is particularly crucial to prevent recontamination of surfaces. This problem can be easily solved if viability experiments are carried out at the same time.

More research has to be carried out in order to determine plasma parameters and conditions that would completely sterilize biofilms. The technology is still somehow expensive compared to other sterilization methods. However, as most of those methods are ineffective towards biofilms or cannot be applied to all circumstances, the use of plasma still offers many promising opportunities for application. So far, results have been obtained with some model organisms but most of the naturally occurring biofilms are mixed populations. In years to come, we expect to see more research on mixed biofilms and the development of plasma applicators with different geometries.

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A sub-microsecond pulsed plasma jet for endodontic biofilm disinfection

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Résumé

The antimicrobial effect of a non-thermal plasma jet, employing sub-microsecond pulses, against endodontic biofilms, was studied with an *in vitro Enterococcus faecalis* biofilm model and a pilot *ex vivo* saliva biofilm model. The studies are to assess the application feasibility of the plasma for root canal disinfection.

Abstract

A pulsed, tapered cylindrical plasma jet, several centimeter long and <2 mm in diameter, has been generated by a concentric tubular device for root canal disinfection [1]. This plasma dental probe is typically powered with ~100 ns, 1-2 kHz, multi-kilovolt electric pulses and filled with 1-5 SLPM (standard liter per minute) He/(1%)O_2 flow. The energy per pulse was measured to be 1.2 – 1.8 mJ, resulting in less than 2 W average power when the plasma device operates at 1 kHz [2]. This low power, pulsed, plasma jet consists of ionization fronts propagating away from both electrodes at speeds of the order of 10^7 cm/s [3]. Optical emission spectroscopy reveals that reactive plasma species including O and O_3 are present and may play significant roles in the bactericidal process [1, 4]. We report here an *in vitro* study of the antimicrobial effect of the room temperature plasma jet against monolayer *Enterococcus faecalis* biofilms on bovine dentins. Resultant colony-forming unit counts were associated with changes in bacterial cell morphology observed using scanning electron microscopy (SEM) following the treatment and control. Treatment of dentin discs cultivated with *E. faecalis* monolayer biofilms with the plasma (average power ≈ 1W) for 5 min resulted in 92.4% kill ($P < 0.0001$). Severe disruption of the cell membranes were observed for the plasma treatment group, while the morphology of the cells remained intact for the negative control group, as shown in Fig. 1. In addition, a pilot *ex vivo* test was also conducted to examine the bactericidal effect of the plasma against saliva-derived biofilms cultivated in human root canals. Fig. 2 demonstrates the plasma jet impinging into a root canal. Plasma treatment of two root canal specimens for 5 min resulted in removal of the biofilms for a depth of 1 mm in the root canal of one specimen, and only spottily cleared dentinal surfaces occupied with physically disrupted bacterial biofilms for the other specimen. Improvement of the disinfection results is in progress through the optimization of the technology that will enhance the bactericidal effect and deliver the plasma jet into the entire root canal. In summary, the antimicrobial effects of the plasma jet against single or multi species biofilms on dentinal surfaces or in root canals have been demonstrated. We conclude that this non-thermal pulsed plasma-based technology is a potential alternative or supplement to existing protocols for root canal disinfection. This work is supported by a grant from the National Institute of Dental and Craniofacial Research (NIDCR), one of the National Institutes of Health (NIH) in the U.S. Department of Health and Human Services.

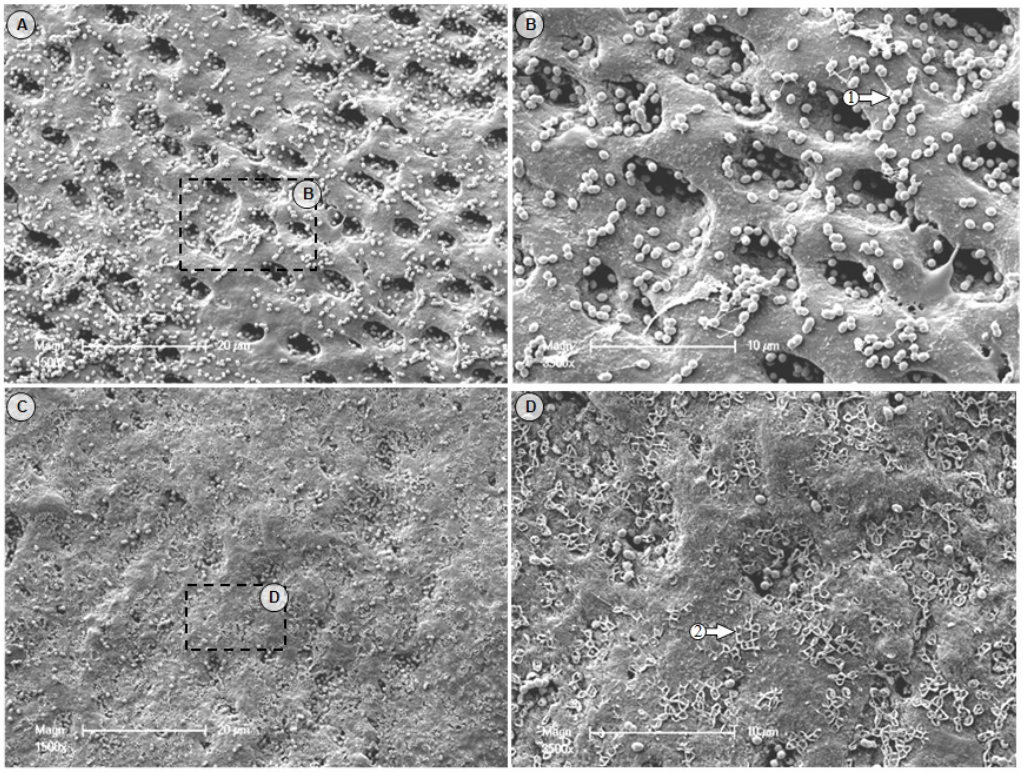

Fig. 1: SEM: (A) monolayer *E. faecalis* biofilms cultivated on bovine dentin discs without treatment (the negative control); (B) the same monolayer biofilms at higher magnification (3500x): *E. faecalis* cells appear morphologically intact (arrow 1); (C) after the plasma treatment (He/(1%)O_2 plasma, 5 SLPM, 5 min); (D) the same plasma treatment group at higher magnification (3500x): membrane integrity severely compromised or damaged cells were mostly observed (arrow 2).

Fig. 2: The room temperature plasma jet impinging into a root canal

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Inactivation of microorganisms in model biofilms by atmospheric pressure non-thermal plasma

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Résumé

Microorganisms have high ability to attach strongly to any surface and form biofilms. Biofouling can lead to corrosion of metallic constructions and decrease essentially the lifetime of wood and concrete facilities. Drawbacks of the traditional methods for microorganism inactivation and biofilm destroying stimulate a development of novel approaches to the protection against biofouling and biodamage processes. This paper reports fresh results on sterilization of microorganisms including biofilms with usage of non-thermal plasma.

Introduction

Protection of industrial facilities, devices, materials, etc against the biofouling and biodamage (including biocorrosion) is one of the great challenges of a modern science and industry. This problem is rather difficult to resolve since biofilm-forming microorganisms are highly resistant to biocides. For instance, sterilization of the waterworks requires too high dose of biocides that is harmful from environmental point of view. Additionally, water treatment by biocides takes too long time (more than twenty-four hours).

Here, we are dealing with so-called non-thermal plasma (NTP) sterilization of microorganisms including biofilms. NTP sterilization can lead to the eventual abandonment in usage of heat and the chemically aggressive, toxic and environmentally harmful liquid and gaseous agents. Our previous promising results on this topic are published in [1]. Present report contains results on atmospheric pressure NTP inactivation of microorganisms in model (monoculture of *Escherichia coli* and *Bacillus subtilis*) biofilms.

Biofilms are spatially and metabolically structured microbial communities within extracellular polymeric matrix at the phase interface. Biofilms distinguish themselves with a specific dynamics of the growth and substrate digestion modes, and are highly resistant to chemical biocides. A simple and convenient research model of a biofilm is pure culture of colonies grown on agar.

Types of microorganisms used

In our work we used two model biofilms generated by *E. coli* and *B. subtilis* monocultures. We studied the biofilms grown on the agar and on the surface of inert carriers (mild steel and polypropylene coupons) immersed in starvation (poor) medium. Biofilms grown on the agar are convenient to work with, but they are too simplified models of natural biofilms. Model biofilms grown on the surface of inert carriers are much closer to natural biofilms.

We treated so-called “young” biofilms grown overnight, and “old” biofilms grown for several days. The total number of microorganisms constituting *E.coli* biofilms grown in enriched medium reached 10^{11} CFU as early as overnight. As biofilms were getting old, their number did not change. But the biofilms increased in diameter, while their superficial density decreased (from 2.9×10^{9} CFU/mm^2 for a biofilm grown overnight to 3.6×10^{8} CFU/mm^2 for a three days old biofilm).

Results

After plasma processing, the biofilm grown overnight was found to be most susceptible, whereas the biofilm grown for three days was less susceptible. The decreased susceptibility of the “old” biofilm is likely associated with physical “ageing” of the biofilm microorganisms that leads to the cell division inhibition, whereas a pool of reserve cellular substances, as well as strength of the cell wall, increases. Besides, the “old” biofilms may contain a significant amount of the dead cells screening the living cells against plasma action.

Treating *E.coli* metal and plastic coupons with low-temperature plasma showed full inactivation of microorganisms of biofilms, with the majority of cells being killed within the first minute of the treatment (Fig.1). The biofilm grown for 3 days on a metal coupon appeared to be the least susceptible.

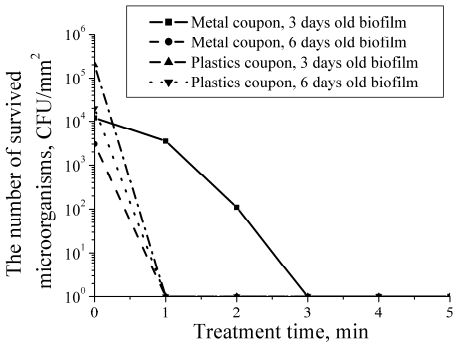

Fig. 1: The rate of survived *E.coli* age-varying biofilms grown on metal and plastic coupons.

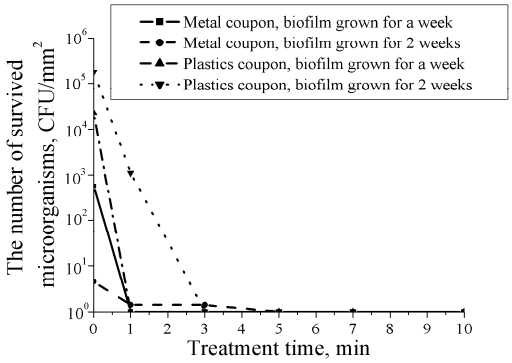

Fig. 2: The rate of survived *B.subtilis* age-varying biofilms grown on metal and plastic coupons.

Research on *B.subtilis* biofilms grown on both types of coupons has shown that these biofilms are more tolerant than *E.coli* biofilms (Fig.2). Although the initial titer of *B.subtilis* biofilms was lower than that of *E. coli* biofilms, they became inactivated in 3-5 minutes of the treatment. Besides, in contrast to *E.coli* biofilms, *green* (one week old) *B. subtilis* biofilms appeared more susceptible than two weeks old biofilms, probably due to the transition of some *B. subtilis* cells from vegetation to spores.

To clarify the mechanism of NTP-cell inactivation, we have done the experiments related to checking the integrity of some individual cell structures (cell wall and membrane) after plasma action. The method is based on measuring the number of free intracellular nucleotides released by a cell in response to the NTP exposure (2-10 min). Cells were precipitated by centrifugation (5810R *Eppendorf*; 12000 rpm) for 20 min. A concentration of supernatant nucleotides was determined from measurement of optical density of a liquid at $\lambda = 260$ nm by the spectrophotometer (*Shimadzu* UV-1700).

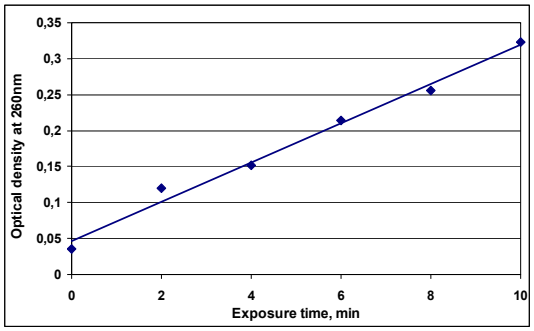

Fig. 3: The dynamics of free nucleotides concentration in supernatant vs NTP-exposure of *E.coli* cell suspension in the isotonic conditions.

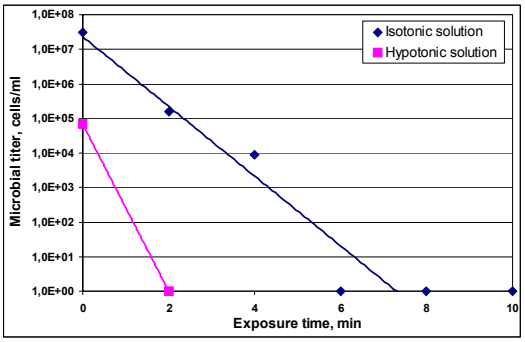

Fig. 4: Survivability of NTP-treated *E.coli* liquid culture that was incubated subsequently in the osmotic pressure-varying solutions.

Comments to Fig.3: Free nucleotides (mainly low-molecular-weight ones) appeared in the supernatant because of partial or complete degradation of both the cell wall and cell membrane. So cold plasma treatment brokes partially or fully the integrity of the cell wall and cell membrane and could kill thereby the cells.

Comments to Fig.4: The NTP-exposure results in partial or complete degradation of both the cell wall and cell membrane that leads to loosing the cell wall strength. After that, the cells with a depressed wall strength can be easily burst in hypotonic solution because of huge turgor pressure.

One can conclude that plasma sterilization procedure differs beneficially from conventional methods to control biofilms. No chemically aggressive reagents are required, and the plasma procedure takes short time. Owing to these features the NTP-procedure is expected to be widely applied in different areas where control and inhibition of biofilm growth are urgent (pipelines, surfaces of stone, wood and concrete buildings, etc).

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Non-thermal plasma treatment of dentin surface for bacterial disinfection and improved composite restoration

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Résumé

The objective of this study is to investigate the treatment effects of non-thermal atmospheric gas plasmas on dentin surfaces for oral bacterial disinfection and composite restoration improvement. Oral bacteria of *Streptococcus mutans (S. mutans)* and *Lactobacillus acidophilus (L. acidophilus)* with an initial bacterial population density between 1.0 x 10^8 and 5.0 x 10^8 cfu/ml were seeded on various media and their survivability with plasma exposure was examined. The plasma exposure time for a 99.9999% cell reduction was less than 15 seconds for *S. mutans* and within 5 minutes for *L. acidophilus*. To evaluate the dentin/composite interfacial bonding, extracted unerupted human third molars were used by removing the crowns and etching the exposed dentin surfaces with 35% phosphoric acid gel. The teeth thus prepared were sectioned into micro-bars as the specimens for tensile test. Student Newman Keuls (SNK) tests showed that the bonding strength of the composite restoration to peripheral dentin was significantly increased (by 64%) after 30 s plasma treatment of the dentin surfaces. The findings from this study indicated that non-thermal atmospheric plasma technology is promising for dental clinical applications.

Introduction

Polymethacrylate-based dental composites have received widespread clinical acceptance as alternative restorative materials to dental amalgam amid concern regarding the potential health risks associated with mercury released from dental amalgam. As supported by results from multiple clinical and laboratory studies, however, the current dental composite restorations suffer from much reduced longevity mainly due to interfacial failures, which cause microleakage, sensitivity, recurrent caries, and composite restoration failure.[1,2] The interface failure of composite restoration resulted from adhesive bonding failure of the dental adhesive/composite to the surrounding tooth structure. Adquate dentin/adhesive bonding requires dispersion of the adhesive throughout the dentin surface and micromechanical interlocking of adhesive with collagen fibrils in decalcified dentin.[3] In this study, non-thermal atmospheric plasmas was used to prepare and engineer dental surfaces and study the plasma treatment effects on dentin surfaces in terms of bacterial disinfection, dentin surface modification, adhesive wettability improvement, interfacial bonding enhancement. Effective plasma disinfection of oral bacteria will help save healthy dental tissues that are often extensively removed in clinical practice by mechanical drilling to ensure complete removal of caries-causing microorganisms.

Experimental

A recently developed atmospheric cold plasma brush (ACPB)[4,5] was utilized to clean/disinfect oral bacteria and prepare dentin surfaces for dental adhesive and dental composite application. Two kinds of caries-causing bacteria, *S. mutans* and *L. acidophilus,* were seeded on various media and their survivability with plasma exposure was examined. Extracted unerupted human third molars were used and the occlusal one-third of the crown was sectioned by means of a water-cooled diamond saw. The exposed dentin surfaces were polished with 600 grit SiC sand papers and then etched using 36% phosphoric acid. Adaper™ Single Bond Plus dental adhesive and Filtek™ Z250 composite (3M ESPE Dental Products, USA) were applied and light cured as directed. Dentin/composite bars (8-10×1×1 mm) were cut from the prepared teeth for micro tensile testing and interface characterization.

Results and discussion

Figure 1 shows the cell surviving curve of *S. mutans* with different plasma exposure time on three different supporting media. It can be seen that the argon plasma brush is very effective in killing *S. mutans*

and a very short plasma exposure time of less than 15 s gave a complete kill of the bacteria. It was also found that a longer plasma treatment time of 5 min was required for completely killing *L. acidophilus*. To investigate bacterial cell damage caused by the plasma treatment, a UV-visible spectrometer was used to monitor the absorbance peak intensities at wavelengths of 260 nm (DNA absorbance) and 280 nm (protein absorbance). For both bacterial samples, a longer plasma exposure did increase the peak intensity of absorbency at wavelengths of 260 nm and 280nm, which suggest the leakage of protein and/or nucleic acids from the bacterial cells due to the cell membrane damages upon plasma exposure. The plasma-induced cell membrane damages were further confirmed by SEM.

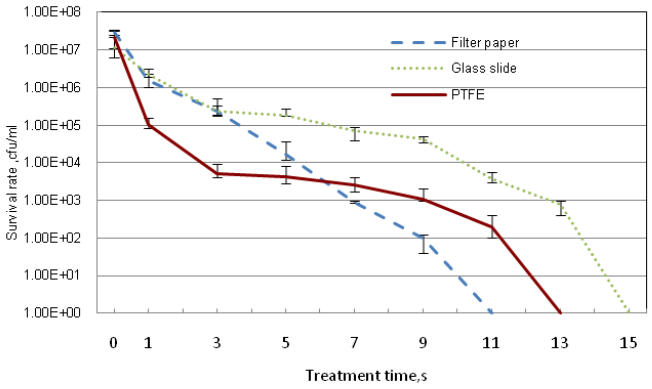

Fig. 1 : Plasma disinfection effect on *S. mutans*. Plasma conditions were 2000 sccm Ar and 10 W DC Power input.

FTIR surface analysis showed structural changes in the surface of the demineralized dentin after plasma treatments. A new shoulder peak around 1760 cm^{-1} associated with carbonyl stretch was found and an amide II shift of ~10 cm^{-1} was observed (1543 cm^{-1} before to 1533 cm^{-1} after), which might indicate the secondary structural changes of dentin collagen after plasma treatment. Figure 2 shows the statistical comparison of ultimate tensile strength data obtained with test specimens prepared from plasma treated dentin and the untreated controls. Statistically significant differences in tensile strength between all specimens using SNK method were observed. A significant difference was found between the peripheral dentin that was plasma treated for 30 s and all the other treatments. SEM images of the fracture surface generally showed that more composite remained on plasma treated dentin surfaces when compared with the controls, indicating much enhanced dentin/composite interface bonding.

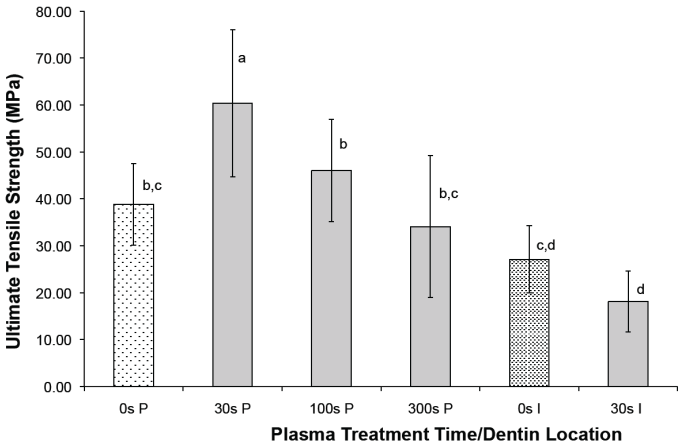

Fig. 2: Statistical comparison of ultimate tensile strength obtained with test specimens prepared from plasma treated dentin and the untreated controls (0s P and 0s I). P: Peripheral dentin, I: Inner dentin; Different letters indicate statistically significant differences (α = 0.05).

Conclusions

Our experimental results showed that atmospheric plasma treatment was very effective in cleaning/disinfecting caries-causing oral bacteria. Plasma surface preparation of dentin could significantly increase the dentin/adhesive interfacial bonding strength. The findings from this study indicated that non-thermal atmospheric plasmas could be a promising technique in dentistry for many clinical applications such as bacterial disinfection, caries early prevention, and improved composite restoration.

Acknowledgements

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Xenon iodide exciplex lamp as an efficient source for the UV surface cleaning and water decontamination

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Résumé

In this study a good cleaning effect of glass surface and sterilization action was achieved with the DBD-driven XeI* exciplex lamp. The main part (~76%) of the lamp output was due to the $B \rightarrow X$ transition of XeI* exciplex at 253 nm. The contribution of an atomic iodine emission in the range of 178–207 nm has been confirmed. Germ reduction experiments with the XeI* excilamp have been carried out in a water flow reactor.

Introduction

Conventional technology for disinfection by UV irradiation is based on low-pressure mercury lamps. The development of a new mercury-free UV lamp is very important due to the environmentally unfriendly nature of mercury. The excilamps (excimer or exciplex lamps) based on mixtures containing xenon and iodine vapours emitting mainly due to XeI($B^2\Sigma_{1/2} - X^2\Sigma_{1/2}$) at $\lambda = 253$ nm are considered to be the efficient sources in the UVC (200–290 nm) range. Recently, the sterilization action of a DBD-driven XeI* excilamp operated with a Xe/I_2 mixture has been reported for the inactivation of *Bacillus Subtilis* spores in a steady state mode [1]. In this study, we report the investigation of XeI* excilamp operated with the Xe/I_2 mixture and using it for the UV surface cleaning and UV inactivation of *Bacillus Subtilis* spores in a water flow reactor.

Results and discussion

In these experiments we used the dielectric barrier discharge (DBD) lamp in coaxial design consisting of two quartz tubes and electrodes, which were applied to the inner surface of the smaller tube and the outer surface of the outer tube [1]. The exciplex lamp was excited using a custom built power supply, producing bipolar pulses with peak-to-peak voltage $U = 0$–4.4 kV, $f = 21.5$–115 kHz, provided the highest XeI* UV output at a frequency of 60 kHz. The emission spectra in the VUV–UV range (170-400 nm) were measured with the VM-502 0.2 m vacuum monochromator (Acton) equipped by a PMT R928 (Hamamatsu) with a scintillator. The radiation power of the excilamp in absolute units was measured by a UV power meter C8026 (Hamamatsu Photonics K.K.) equipped with a calibrated H8025-254 sensor head (210–350 nm).

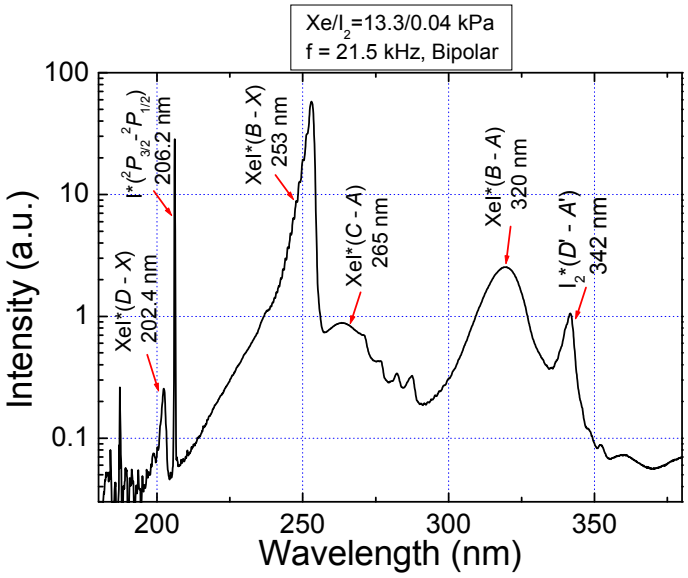

Fig. 1: UV emission spectrum of the XeI* excilamp. Y axis is in a logarithmic scale in order to show the low-intensity features.

The emission spectrum of the XeI* excilamp, operated with a Xe/I_2 = 13.3/0.04 kPa mixture, in germicidal region (180–300 nm) includes atomic iodine I* radiation in the range of 178–188 nm (178.3, 179.9, 183.0, 184.4, and 187.6 nm) and at λ = 206.2 nm, XeI*($B \rightarrow X$, $\lambda_{max} = 253$ nm), XeI*($C \rightarrow A$, $\lambda_{max} = 265$ nm) exciplex emission, weak traces of XeI*($D \rightarrow X$, λ_{max} = 202.4 nm) and I_2*($F \rightarrow B$) in the range of 270–280 nm (Fig. 1). Moreover, the XeI($B \rightarrow A$, $\lambda_{max} = 320$ nm) and I_2($D' \rightarrow A'$, $\lambda_{max} = 342$ nm) emission was also registered. The radiation of the atomic iodine and XeI*($B \rightarrow X$, $C \rightarrow A$) coincides with the absorption maxima of DNA nearly at 200

and 260 nm so it can be efficiently used for UV sterilization. It should be noted that very often a sterilization efficiency curve is shown with one maximum around 260 nm mainly due to the small penetration depth in water for UV emission with λ<220 nm. However, the main target for the UV sterilization is DNA and the short wavelength part (175–210 nm), which is transparent for air and the quartz bulb of the lamp, can be efficiently used in the case of a setup with a thin water layer, and for air or food package sterilization.

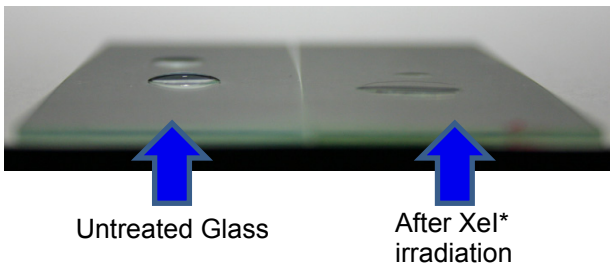

Fig. 2: Glass plates w/o treatment (left) and after 1 min. UV irradiation by means of XeI* exciplex lamp (right).

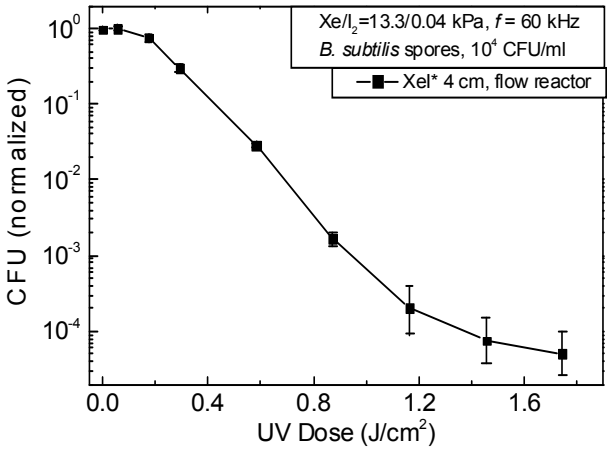

Fig. 3: Normalized number of CFU (*B. subtilis*) as a function of UVC energy from XeI* excilamp. Distance to the lamp: 4 cm, 1 Liter of flowing water.

The glass plates were chosen as the test objects for the UV surface cleaning. It can be seen from the figure 2 that after 1 minute irradiation the water drop on the glass surface has a contact angle about three times less in comparison with the untreated surface. It means, the UV radiation from the XeI* lamp destroyed the oil film on the surface and can be used for the UV cleaning.

Figure 3 shows the changes in the normalized number of CFU in the water flow reactor as a function of the cumulative UV dose of XeI* exciplex lamp, emitted during the UV treatment. One experiment was carried out when the volume of irradiated water was covered with a plate preventing the penetration of foreign microflora from outside, and another one – without a cover. Almost the same CFU reduction was observed in both measurements. The higher UV doses, needed for inactivation of *B. subtilis* spores in the water flow reactor in comparison with the steady state mode [1], can be explained by the short reaction time. This is also the possible reason for the presence of a threshold at about 0.1 J/cm^2 in CFU reduction. Taking into account the water flow circulating velocity, the cross section ratio of the tubing and the water layer on the plate, and the plate length, we can estimate that the total exposure time of the same portion of water is about 49 s for a 30 min irradiation, since the processing time of water per single pass in the water flow reactor is 0.3 s and the same portion is irradiated 162 times during 30 min irradiation. Then, using the data of [1], we can calculate that the UV dose, which reaches the same portion of water, does not exceed 40–42 mJ/cm^2. This is close to the energy range of the steady state mode. Note that it is possible to improve the reactor design placing the lamp along the water stream and using a set of the exciplex lamps. Thus, the latter experiment has shown the possibility to use the XeI* exciplex lamp (or a set of the lamps) for water decontamination in flow systems.

Summary

The sterilization action of the DBD-driven XeI* excilamp was tested on the inactivation of *B. subtilis* spores. A reduction by more than 4 orders of magnitude of CFU in *B. subtilis* spores was achieved in a water flow reactor and the D-value was about 0.4 J/cm^2. An additional effect of the I* radiation at 206 nm and in the VUV range (178–188 nm) was confirmed. This research demonstrates that the DBD-driven XeI* excilamp can be used for the UV cleaning and inactivation of microorganisms not only in a steady state mode, but also in movable systems (drinking water treatment or food package sterilization).

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Characterization of bacterial and bio-macromolecule damage by (V)UV and particle channels of X-microscale atmospheric pressure plasma jet (X-µAPPJ)

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Résumé

Atmospheric pressure plasma jets effectively inactivating bacteria on surfaces including infected tissues. This is due to the combined effects of (V)UV radiation, reactive oxygen and nitrogen species, ions, or high electric fields. Here we present how (V)UV radiation and reactive radical species produced by a micro scale atmospheric pressure plasma jet can be separated by a lateral He flow steering the heavy particles inside the effluent to facilitate the study of mechanisms leading to bacterial cell death. The new jet geometry is called X-jet. The densities of reactive species can be compared to the well characterized geometry of the µAPPJ [1]. We show that heavy particles are steered away by the additional He flow effectively by comparing etching of a-C:H film and inactivation of vegetative *Escherichia coli* and *Bacillus subtilis* cells after exposure to the (V)UV or particles channel. Additionally, by tuning the jet parameters we can select conditions in which either O radicals or O_3 ozone molecules are the dominant species reacting with the biological sample. The results of treatment of bio-macromolecules will be presented here. DNA, RNA and proteins are all damaged by plasma treatment.

Introduction

Atmospheric pressure plasmas are known to be capable of inactivating bacteria. The inactivation is, however, usually only qualitatively studied and the exact inactivation mechanisms and the role of different reactive species (oxygen radicals, metastables, UV photons, ions) and possible synergistic mechanisms among them are not well understood. We use a radio frequency driven atmospheric pressure plasma jet operated with He gas or He/O_2 gas mixture to treat vegetative *E. coli* and *B. subtilis* cells. *E. coli* is a Gram-negative bacterium that is commonly found in the lower intestine of warm-blooded organisms and *B. subtilis* is a Gram-positive bacterium that serves as a model organism for Gram-positive pathogens. The plasma is generated in a square 30 mm long channel with an area of 1x1 mm^2 formed by two electrodes and two glass plates. This source is very well characterized by laser spectroscopy, optical emission spectroscopy, and mass spectrometry. The fluxes of oxygen atoms, ozone molecules, and UV, and VUV photons are known. It is possible to suppress or enhance the flux of the selected species by proper selection of operating parameters and by modification of the source geometry. Additionally, we have modified the exit nozzle of the jet in such a way that a second flow of He gas, which crosses the plasma effluent, steers heavy particles from the jet axis as shown in Fig. 1. We call

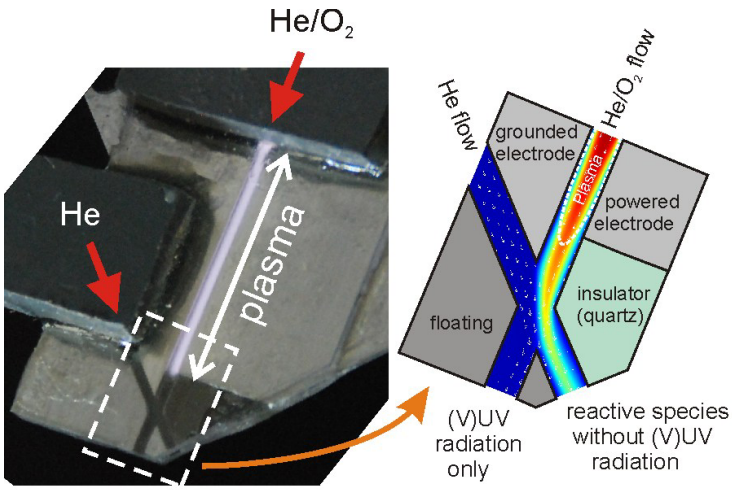

Fig. 1: Left: Photograph of an atmospheric pressure microplasma jet in He/O_2 gas mixture with an additional He flow crossing the plasma effluent. The additional helium flow steers the flow of radical species into a side channel. (V)UV radiation propagates along the line-of-sight with the plasma. Right: fluid dynamics simulation of the flow in the X-Jet. Arrows correspond to gas velocity and the color map represents concentration of reactive species (e.g. O atoms; scale increases from blue to red). This simulation illustrates the separation of (V)UV and heavy reactive particles.

this modified jet X-jet. The (V)UV radiation propagating on the line-of-sight is then effectively separated from heavy particles. The effect of these plasma components, (V)UV radiation and reactive heavy particles on the *E. coli* and *B. subtilis* cells can be studied separately. This is demonstrated in Fig. 2, which shows inhibition zones of *B. subtilis* vegetative cells on agar medium produced by the X-jet without and with additional He flow after 1 and 6 minutes of treatment. Without the steering He flow, a typical dose-effect relationship could be observed. Elongating treatment time resulted in increasing inhibition zones. Sample treatment with activated steering flow resulted in two separate inhibition zones. The upper inhibition zone exposed to the effluent of the direct channel was always smaller than the lower inhibition zone exposed to the effluent of the crossed channel. No inhibition zone(s) could be observed after 1 minute of plasma treatment indicating higher efficiency of the combined treatment. The microplasma jet and its X-jet modification are also characterised by means of molecular beam mass spectrometry (MBMS) in similar ways to those described in [1]. Inactivation efficiencies are also compared.

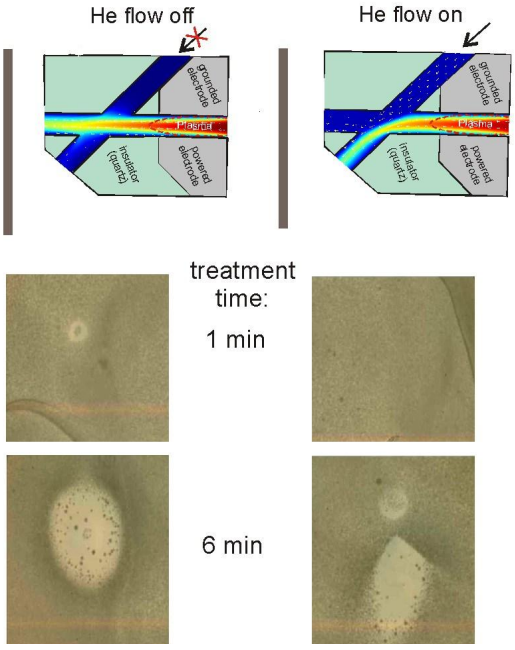

Fig. 2: Inhibition zones after treatment of *B. subtilis* without and with additional He flow. Treatment geometry as indicated at the top.

Besides treating whole bacteria, plasma impact on bio-macromolecules is investigated. DNA-, RNA-, and Protein damages might play an important role in bacterial inactivation. The different damaging effects between (V)UV and particles are investigated and compared thanks to the X-jet. Plasma not only induces single and double strand breaks into DNA, it is also capable of cross-linking several DNA fragments into plasmid dimers or multimers (Fig. 3a), as well as inducing different kinds of nucleotide specific alterations. In contrast, RNA is mostly damaged via the induction of single strand breaks or by plasma based etching (Fig. 3b). The latter also applies to plasma induced protein damage. Nucleotides were dried and treated on glass slides, washed and analyzed via agarose gel electrophoresis. Etching experiments with BSA were observed using a laser scanning microscope and compared to the typical etching model of an a-C-H coating to differentiate between etching impact on a biological sample compared to non-biological materials.

With the help of the X-jet, we are able for the first time to analyse damaging effects of different effluent components under *in vivo* and *in vitro* conditions. We will further investigate plasma impact on bio-macromolecules, as these have a huge impact on clinical and industrial applications and will further deepen our understanding about the basic bacterial inactivation mechanisms.

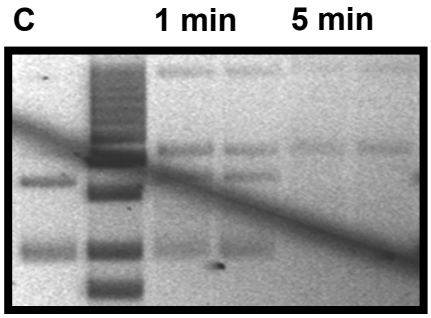

Fig. 3a: Dried pUC18 plasmid DNA treated with undivided effluent. Control (C) shows supercoiled and relaxed (lower and upper band respectively) plasmid form. After plasma treatment, multimeric and dimeric plasmid forms occur.

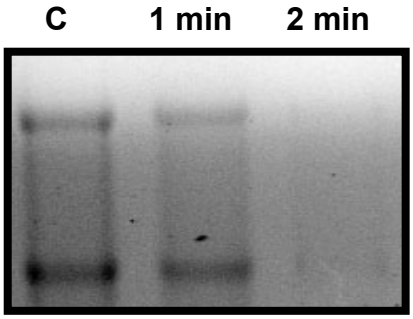

Fig. 3b: Total *E. coli* RNA treated with undivided effluent. Loss of intensity of distinct 23S und 16S rRNA (upper, lower band respectively) indicates etching and

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POSTERS

Resistive barrier discharge device to generate gas plasma for food decontamination

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Résumé

The present study intends to analyse the superficial decontamination power of gas plasma generated at atmospheric conditions by a Resistive Barrier Discharge (RBD) device. Different atmospheric conditions and treatment times were considered. The plasma oxidation power was assessed by measuring the absorbance associated with the primary and secondary oxidation products of sunflower oil. Moreover, the inactivation of natural microflora and some pathogens on the outer skins of fresh fruit and surface of the shell eggs was investigated. Main results indicated that the treatment time was positively correlated to the primary oxidation products, while the Relative Humidity levels showed to play a role in the production of the secondary ones. The efficiency of plasma treatment to decontaminate both fresh pears and table eggs was highly dependent on the exposure time and RH values.

Introduction

The decontamination efficacy and mechanism of the gas plasma towards different type of microorganisms together with the techniques able to generate the ionized gas at atmospheric conditions were widely investigated [1, 2, 3]. The possibility of achieving the decontamination at low levels of temperature and pressure makes the technique promising for the superficial treatment of food products. The gas plasma potentiality was confirmed by the results of some recent researches conducted on the main microorganisms infecting fruits [4], vegetables [5], grains and legumes [6] and shell eggs [7]. In order to study the optimal treatment conditions, the present work intends to analyse the gas plasma produced by an RBD device in terms of oxidative power (towards sunflower oil samples) and decontamination efficacy (towards fruit and shell eggs).

Materials and methods

The analysed gas plasma was generated between three pairs of parallel plate electrodes made of brass (one of the two electrodes was covered by a 5 mm thick glass sheet used as high resistive materials to prevent arc) (Fig.1). The voltage at electrodes was produced by three high voltage transformers and power switching transistors. The maximum volume of the treatment chamber was about 70 dm^3 and the generated plasma species were driven towards the product by three fans. Electrical and chemical characterization of the gas plasma produced by the above described device were conducted in a previous work by Ragni et al., 2010 [7]: with an input DC voltage of 19V, the discharge was characterized by a potential difference of about 15kV, while the analysed emission spectrum showed, as expected with the air gas, the formation of very reactive species such as the positive ion N_2^+ and NO and OH radicals.

In order to understand the power of the oxidative species generated during treatments characterized by three different durations (30, 60 and 90 min) and two different levels of the relative humidity (RH= 35% and 65%, 21°C), oxidative tests were conducted with sunflower oil samples (10 ml each placed in a Petri dish). The absorbance for conjugated dienes and trienes in control (treatment time= 0) and oxidized samples were measured at 232 nm and 270 nm, respectively [8].

The decontamination efficacy was evaluated on fresh pears and shell eggs containing only the indigenous microflora or deliberately contaminated with pathogens (*Salmonella* Enteritidis, *Escherichia coli* and *Listeria monocytogenes*). Food products were exposed to gas plasma for 0, 10, 20, 30, 45, 60 and 90 min at 35% and 65% RH values, and the surviving cells of the target microorganisms were enumerated by plate countings onto unselective and selective media.

Results and discussions

The oxidative behaviour of the sunflower oil showed that the absorbance associated to the primary oxidation products content significantly increased by increasing the treatment time of exposure. However, this content was not correlated with the relative humidity of the discharge atmosphere. The absorbance associated to the secondary products significantly decreased among treatment times, and the relative humidity showed to play, although not always clear, a role in the production of conjugated trienes (their decrement was often significantly lower for the samples treated with highest RH values).

Results of the microbiological analysis showed that gas plasma was effective against both natural microflora and target pathogens. In general the decontaminating efficacy was enhanced by increasing the treatment time and in the presence of humid air. Surface reductions by 1.5 and 2.5 log units/fruit of total spoilage flora and moulds, respectively were observed in about 45 minutes of treatment at 65% RH for fresh fruit.

Maximum cell reductions ranging between 1.5 and 4.5 log units/eggshell depending on the microbial species were achieved for table eggs following the longest treatment. During a 50-day storage at 25°C cell loads of both control and treated eggs decreased down to undetectable levels. However, such a viability loss was much faster in gas plasma treated ones.

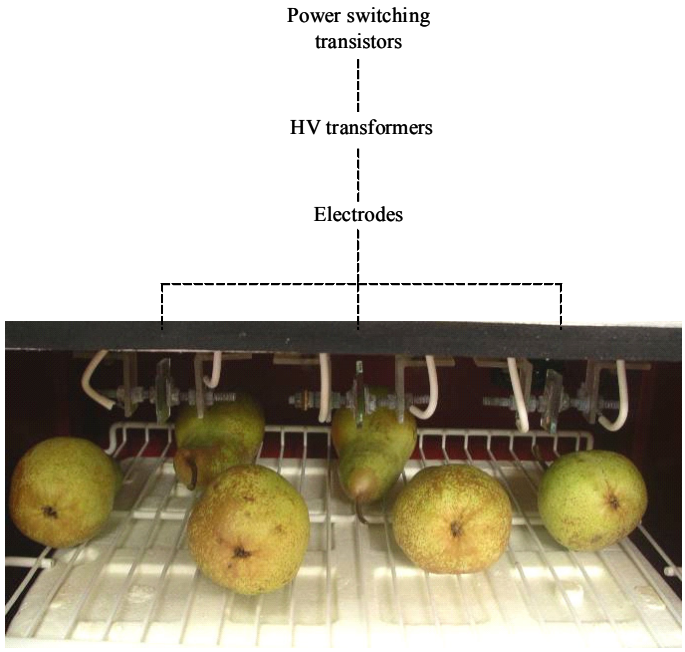

Fig. 1: Particular of the electrodes of the Resistive Barrier Discharge device during the superficial treatment of *Abate* pears.

Conclusions

The RBD gas-plasma prototype proved to generate a low temperature after-glow gas mixture able to significantly reduce the native flora and the inoculated pathogens on the surface of fresh pears and shell eggs. About the oxidation power, further investigations on the transformation of primary to secondary oxidation products should be carried out to clarify also the gas plasma role in the possible food quality modifications.

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Determination of effective UV/VUV radiation of a low pressure inductively coupled plasma for sterilization of spores

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Résumé
Radiation is an important sterilizing agent of a low pressure plasma. Determination of effective wavelength ranges is a crucial step in designing plasma sterilization machines. This contribution focuses on linking a wavelength range to a sterilization efficiency.

Introduction

Plasma sterilization of thermolabile implants is of growing interest as toxic sterilants such as ethylene oxide are tried to be avoided. Additionally residual biomolecules on used medical tools is an indispensable problem. Residuals like lipopolysaccharides can lead to a septic shock, and prions to Creutzfeldt-Jakob disease. Plasma sterilization provides a process which combines sterilization and decontamination.

Experimental setup and results

A low pressure double inductively coupled plasma reactor (DICP) is used for the investigation of the removal of spores [1]. Argon, nitrogen, oxygen and hydrogen are used at a pressure of 5-20 Pa. Rf-power up to 5 kW can be fed through two coils, one at the bottom and one on the top of the reactor. Langmuir probe measurements yield in spatially resolved electron temperature and density. Emission spectroscopy is performed with a VUV- and an Echelle spectrometer. These absolute calibrated spectrometers cover a wavelength range from 112 to 800 nm. A Hiden mass spectrometer measures the density and energy distribution of ions and neutrals.

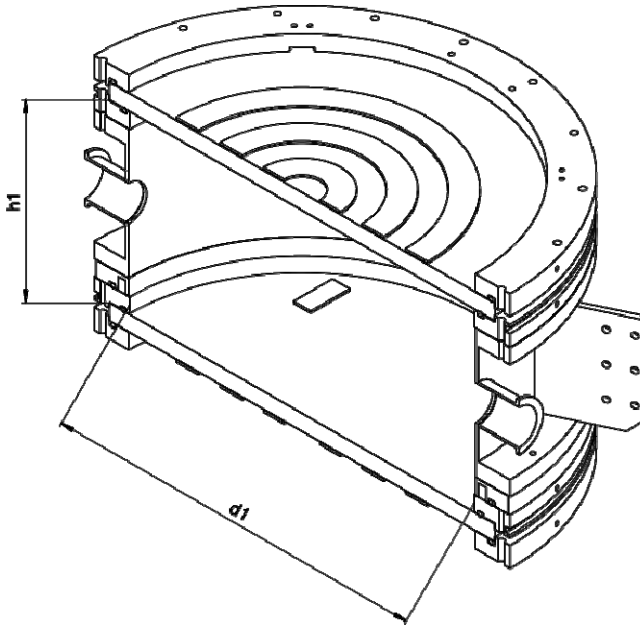

Fig. 1: DICP reactor: d1 = 40 cm, h1 = 20 cm

Different bacterial and fungal spores such as *B. atrophaeus*, *B. subtilis* or *A. niger* are treated at different plasma parameters as well as the spores are covered by different cut-off filters in order to determine the radiation dependency of these spores. Mixtures of different gases allow a selective sterilization of spores in the VUV range. It can be shown that *B. atrophaeus* spores are sensitive to radiation between 235 and 300 nm. In contrast to this *A. niger* spores are resistant to radiation above 235 nm. VUV radiation is needed to sterilize *A. niger* spores.

Acknowledgement

Financial support from the BIODECON (Decontamination of biological systems using plasma discharges) project of the European Union and the support of the Center for Plasma Science and Technology and the Research Department Plasmas with Complex Interactions are gratefully acknowledged.

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Fungicidal and bactericidal effect of plasma and radiowave treatment on biological and medical materials

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Résumé

A germicidal effect of low pressure rf plasma treatment on materials used in medical instruments manufacture has been investigated. An influence of rf plasma and rf electromagnetic field pre-treatments on level of fungal infection of some important agricultural plants has been studied.

Introduction

Plasma and radiowave treatment is widely used for activation and decontamination of surfaces. Nowadays non-thermal plasma is considered functionally, energetically and ecologically as the most efficient tool for pathogenic bacteria inactivation - due to high chemical activity of low-temperature plasma, short-time treatment and minimal thermolabile materials destruction [1–3]. Last years these methods have been successfully applied also in agriculture for seed's sowing quality improvement. It has been shown in a number of previous studies that plasma and electromagnetic field pre-treatments of seeds stimulate their germination and lead to suppression of fungal and bacterial pathogens that cause various plant diseases [4, 5]. A considerable part of the investigations have been performed using 13.56 MHz plasma, and the effect of electromagnetic fields on biological objects has been examined mainly in the microwave (300 MHz–300 GHz) and in the low frequency (50–100 Hz) regions. In this paper, low-pressure capacitively coupled 5.28 MHz discharge plasma as well as 5.28 MHz electromagnetic field treatments are used for plant seeds enhancement and surface decontamination.

Experimental set-up and conditions

Tested species were processed with 5.28 MHz air plasma at pressure 0.5 Torr and were exposed to rf electromagnetic field at atmospheric pressure under conditions of the prebreakdown mode of a capacitively coupled radio frequency discharge operation. The discharge was operated between two parallel round copper electrodes with diameter 120 mm. The distance between electrodes was 20 mm. The investigated samples were placed on the grounded electrode. The supplied full specific rf power was 0.5 W/cm^3. The experimental conditions for magnetic field treatment were as follows: the alternator frequency was 5.28 MHz, the root-mean-square value of magnetic and electric components of the electromagnetic field strength was 590 A/m and 12700 V/m accordingly. The exposure duration of treatments was 5, 10, 15, 20 and 30 min.

To study the peculiarities of microorganisms inactivation on different surfaces in plasma the culture crop emulsion was plotted on a surface of sterile medical products made of polymers, metals (stainless steel or copper) and capillary-porous (surgical suture) samples. The strains of Gram-positive bacteria – *Staphylococcus aureus ATCC 6538* and Gram-negative bacteria – *Escherichia coli ATCC 8739*, clinical isolates of *Staphylococcus - 37C1* and *Enterobacteriaceae EB 158*, as well as spore of *B. subtilis ATCC 6633* were chosen as tested species. Fat-free sterile samples were contaminated in a glass tube containing suspension of microorganisms with concentration of 10^9 CFU/ml prepared according to McFarland turbidity standards. The concentration of microorganisms on spore inoculated products was varied in the range of 10^5-10^7 CFU/ml depending on an effective area. A test of microorganisms survival for treated and control samples was obtained by perform several consecutive dilutions (1:10 in NaCl) of wash-outs from the surface of tested samples (by immersion into a sterile physiological solution). A portion of solution from the most suitable dilution were pipetted into the appropriate culture media and incubated at temperature 37°C for 24-48 hours before the examination.

The effectiveness of pre-sowing plasma and radiowave seed treatments was examined by means of evaluation of the laboratory/field germination ability, seed vitality and a level of fungal infection on sprouting seeds for processed and untreated (control) samples. Seeds of blue lupine (*Lupinus*

angustifolius), honey clover (*Melilotus albus*) and soy were chose for investigations. In the laboratory tests seeds were grown on a moist filter paper in Petri dishes that were kept for 5-7 days (before the first sprout occurrence) in a thermostat at 20-21°C. A plot with the area of 25 m^2 was used for the field test.

Results and discussions

The cultivable cell concentration on the tested surfaces decreased at least by 4-6 orders of magnitude after 20 min of plasma exposure, in dependence on material structure and microorganism genus (fig. 1).

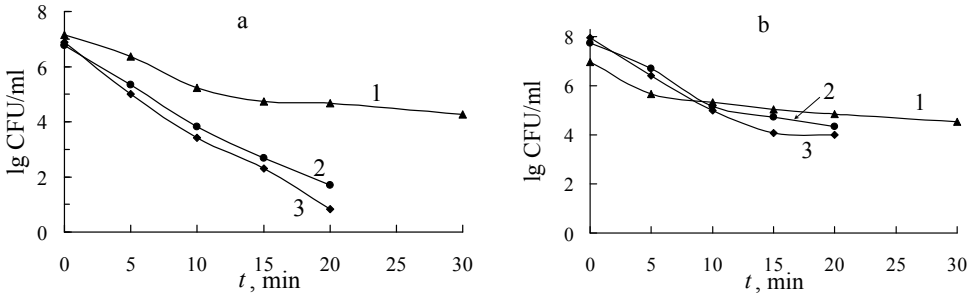

Fig. 1: Concentration of survived cells of microorganisms on metallic (a) and capillary-porous (b) samples in dependence on time of rf air plasma irradiation: 1– spore of *B. subtilis* ATCC 6633, 2 and 3– Gram- positive and Gram-negative bacteria correspondingly.

In the case of samples with a solid surface (polymer, metal) micro-organisms suspension assigned rather evenly on their surface, easily accessible for plasma irradiation. The reason for decrease of plasma sterilizing effect in the case of a porous sample treatment is connected apparently with peculiarities of plasma interaction with porous materials: an initial concentration of microorganisms on capillary-porous samples depended essentially on their surface texture and was not homogeneous upon their surface. It has been shown that Gram-positive bacteria cultures independently of their nature (the museum strains or clinical isolates) demonstrated a greater resistance to the plasma treatment than Gram-negative ones. It may be caused by the basic difference in the form of their cell wall structure: the cell walls of Gram-positive bacteria are made up of twenty times as much peptidoglycan than Gram-negative bacteria that formed together with other proteins a thick outer matrix serve membrane transport regulation and cell expansion of bacteria.

Plasma and radiowave seed pre-treatment during 10-15 min led to decrease (6-14%) of the level of fungal infection caused by *Fusarium oxysporum*, *Alternaria brassicae*, *Stemphylium botryosum* (fig.2).

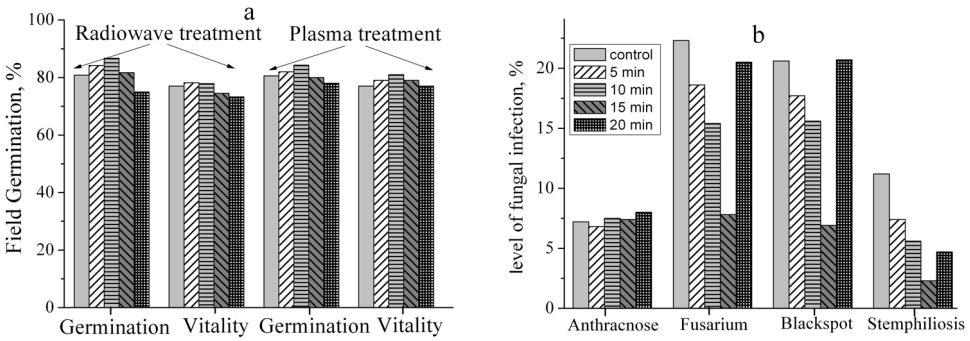

Fig. 2: Field germination capacity of control and treated seeds (a) and a level of fungal infection of blue lupine (*Lupinus angustifolius* L.) (b) as a result of radiowave treatment for different exposure durations.

The same results were obtained for another tested species. It was observed that the increase of treatment duration over 20 min could lead to some oppression of seeds and did not improve plant resistance to fungal infection.

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Biomaterials etching in low pressure inductively coupled discharge

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Résumé

Low-pressure plasma discharges can be applied to remove various biomolecules from surfaces. However, the knowledge of the physical-chemical interaction mechanisms between plasma and biomolecules is still rather poor, which is a major limiting factor for the optimization of this type of plasma treatment.

In the last years several authors presented experimental investigations devoted to isolate potential agents effective in plasma decontamination (UV, radicals, ions, heat) and to identify possible synergic mechanisms between them, but in most cases particle fluxes have been produced outside plasma environments (beam experiments) or the effect on biofilm was studied by physically decoupling the effects of single mechanisms (UV screen, afterglows).

In this work a series of experiments is presented, performed on a double coil planar inductively coupled plasma reactor. Plasma discharge was ignited in oxygen and water vapor containing gas mixtures. These experiments were designed to quantitatively measure the fluxes of different potentially sterilizing species in the plasma phase (ions, radicals, UV and heat) and their interaction effects with a model biofilm (BSA, BrH). Particle fluxes have been calculated using data from Langmuir probe, mass spectrometry, optical emission actinometry and infrared pyrometry measurements.

To understand biofilm removal mechanisms, different experiments have been performed using plasma internal parameters (e.g. fluxes) as independent variables for the decontamination treatments, modyfing one flux component at time while keeping the others constant the influence of synergetic effects between decontamination agents have been measured. Removal rates for biofilms have been measured by means of quartz crystal microbalance used as a quasi-online diagnostic tool in pulsed plasma operation. Profilometry, X-ray photoelectron spectroscopy, atomic force microscope were also used for ex-situ surfaces characterization.

Furthermore the control of the DC bias applied on the sample holder allows changing the energy of the ions (moderate voltages from 10 to 150 V were applied) interacting with the surface.

To understand the interaction, different synergic mechanisms has been postulated and mechanistic model equations for etching rates have been constructed in the well-known context of the Langmuir surface chemical kinetics for plasma-substrate interaction. The results arising from plasma sterilization experiments (ER) have been confronted with the models via linear multiple regression. In order validate a unique model for plasma surface reaction mechanism data fits have been tested rigorously after significant parameter estimates were regressed. A series of non-parametric statistical tests has been applied to determine which model mechanism describes accurately the behavior of the etch rate in our plasma system.

Within the confidence limits of the statistical method implemented for validation, ion assisted chemical etching operating in an ion limited regime proved to be the mechanism which describes more accurately the etching rates for our biological substrates.

Optical emission spectroscopic evaluation of different microwave discharges and its potential application for sterilization processes

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Résumé

The present work aims at studying different microwave flowing discharges containing Ar or N_2 as main carrier gases by Optical Emission Spectroscopy (OES) to demonstrate the potential possibilities of these plasma mixtures to provide O^* and UV species demanded for sterilization purposes at low temperatures. The influence of additional reactants such as NO and/or O_2 is also evaluated. Additionally, some plasma sterilization results with *E. coli* cultures are presented.

Introduction

Plasmas are currently used for the sterilization of heat-sensitive medical tools and the inactivation of bacteria and/or spores. The high energy capabilities at relative low temperatures and costs in conjunction with simple reactor designs make plasmas as a promising alternative to heating or chemical methods (i.e. ovens, autoclaves, EtO oxidation). In recent years, different type of discharges containing inert gases, O_3, O_2, N_2, CO_2, H_2O_2 or mixtures of them have been used with reasonably good results [1-7]. More recent investigations have demonstrated the synergetic role of O* and UV photons [4]. Low-moderated pressure plasmas permit the co-existence of UV photons and atomic atoms what has been reported as a synergetic effect by Moisan et al [1-2]. In contrast, under higher pressures and thus with higher collision frequencies in the atmospheric conditions, a great part of the charged and excited species are recombined with carrier gas atoms.

The aim of the present work consists of a thorough study of microwave (MW) plasma discharges containing different carrier gases such as Ar or N_2 and the influence of the addition of other reactants like NO and/or O_2. These plasma discharges are evaluated by in situ Optical Emission Spectroscopy (OES) in order to identify the main reactive intermediates and the best candidates to provide O and UV species demanded for sterilization process [7-9, 12]. Additionally, some in vitro sterilization results on *E. coli* cultures are shown for selected plasma mixtures.

Experimental

The experimental setup to generate the microwave consists of a quartz tube reactor of 3.5 mm of inner radius with a funnel-type ending and coupled to a surface-wave surfatron launcher which permits the transformation of the electromagnetic power to the travelling wave and the propagation of the plasma discharge (see Figure 1). The MW discharges are produced in a moderated pressure range between 30 and 90 torr, although the presented results are referred to 45 torr. Ar or N_2 were fed through calibrated mass flow controllers as carrier gases with a total flow of 50 cm^3 min^{-1}. The concentration of NO and eventually O_2 were kept constant at 3 x 10^3 ppm and 3x10^4 ppm respectively. Axial profiles along the x-axis of the quartz reactor at different points from the gap discharge have been obtained for Ar-NO-(O_2) and N_2-NO-(O_2) plasma discharges. The OES spectra have been registered by collecting the light with an optical fiber connected to a scanning monochromator (Jobin-Ybon HR250) and a Hamamatsu photomultiplier (R928). *E. coli* DHα were grown in a LB medium at 37 ºC for 24 hours. Cells were washed thoroughly prior to any plasma treatment and the colony forming units were determined by the viable count method [11].

Results and Discussion

The addition of NO into Ar or N_2 yielded different UV emitting species as detected by OES (see Figure 1) thereby indicating different excitation channels for Ar (atomic gas) and N_2 (molecular gas) [7-9]. The presence of Ar also demonstrated to keep plasma discharges stable for a longer range from the resonant

cavity in comparison with N_2. Therefore, Ar sustained plasmas can be considered more suitable for sterilization purposes where the maximum extension of the cleaning chamber should be exposed. The systematic addition of increasing amounts of O_2 was found detrimental in terms of total volume of discharge but positive for the generation of additional O^* radicals and the recombination of dissociated NO^* species. Similar sterilization results were found after comparing Ar-NO, N_2-NO and N_2-O_2 mixtures although slightly superior for the former plasma combination.

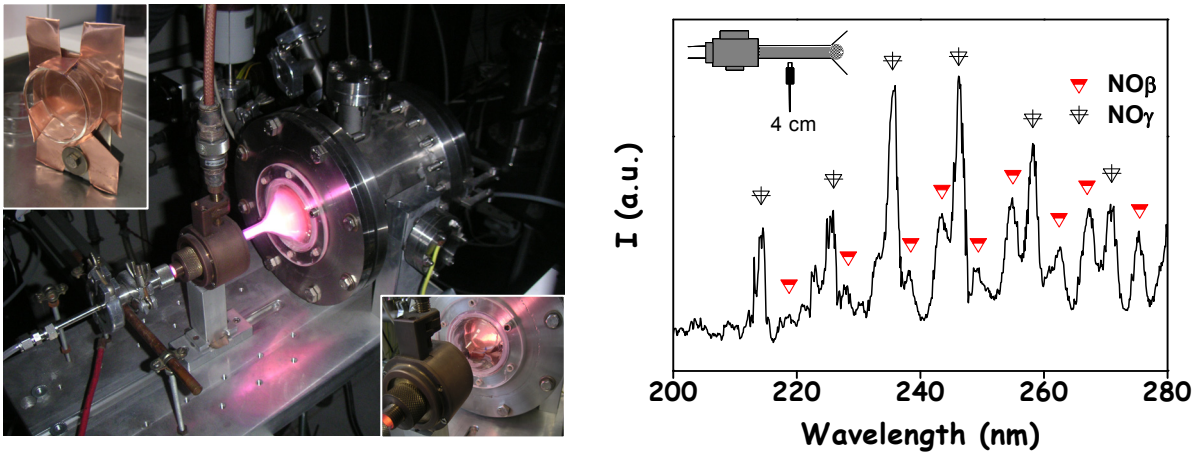

Fig. 1: (Left): Microwave plasma reactor used for the sterilization experiments. The insets show further details about the culture dish holder and its interaction with the plasma discharge generated by the surfatron launcher during an *E. coli* sterilization experiment; (Right): Optical Emission Spectrum corresponding to the UV species generated in a plasma discharge containing Ar-NO.

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Could the addition of agents exceed anti-biofilm plasma efficacy?

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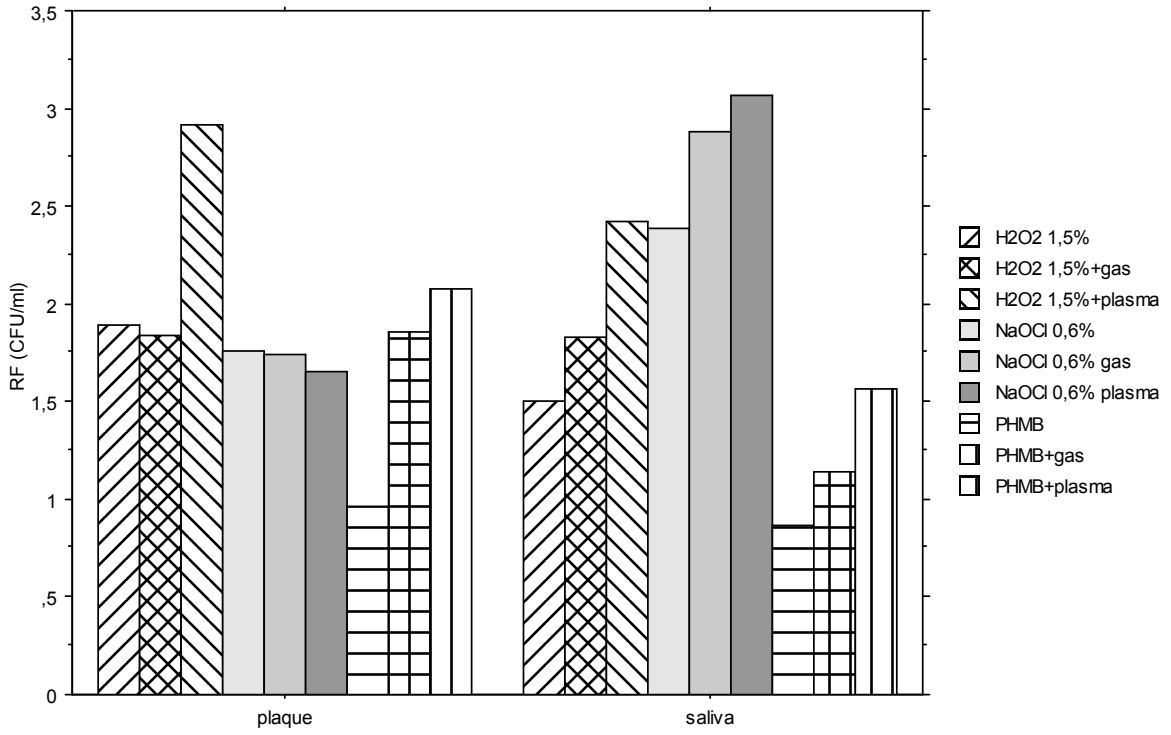
Résumé
The plasma effect against dental biofilms can be exceeded by the addition of some antimicrobial agents. Further research is necessary to specify these effects.

Introduction
Dental biofilms play a major role in the pathogenesis of peri-mucositis. Biofilm removal is a prerequisite for a successful therapy of peri-implant lesions because it could inactivate biofilms [1]. In this study we evaluated the synergistic effect of six antimicrobial agents with atmospheric pressure plasma on three different dental biofilm models.

Material and methods
We assessed the efficacy of kINPen09 argon plasma against monospecies *Streptococcus mutans (S. mutans)*, multispecies aerobe human saliva and multispecies anaerobe plaque biofilm grown on titanium discs *in vitro* in comparison to argon gas. Efficacy of plasma treatment was determined by the number of colony forming units (CFU). The reduction factor (RF, $CFU_{untreated} - CFU_{treated}$) was calculated.

Results
The different biofilm models are differently sensitive to plasma and antimicrobial agents. Plasma showed the highest efficacy against plaque and *S.mutans* biofilms. EDTA+plasma exceeded the effects of plasma treatment in the case of saliva and *S.mutans*. The combination of H_2O_2 and plasma obtained the highest reduction factor. Only in the case of saliva biofilm NaOCl+plasma was more effective than H_2O_2+plasma.

	plaque	saliva	*S.mutans*
gas	1,34	0,42	0,35
plasma	2,16	1,49	2,00
CHX	0,74	0,29	1,78
CHX+gas	1,84	1,38	1,95
CHX+plasma	2,00	1,70	1,95
Oct	1,54	0,79	1,84
Oct+gas	2,65	1,79	1,90
Oct+plasma	1,99	2,79	1,85
EDTA	1,74	0,64	1,11
EDTA+gas	2,02	0,73	1,27
EDTA+plasma	2,08	2,82	2,77



Discussion and conclusion

The plasma effect can be exceeded by the addition of agents. Oxidants like H_2O_2 and NaOCl have the highest effects in combination with plasma but these irrigants are toxic to the oral mucosa [2]. EDTA as a chelating agent could destabilize biofilms and showed a synergistic plasma effect, too without toxic effects to mucosa. Beside octinidine antiseptics could not exceed the pure plasma effect. However, further research is necessary to specify these effects.

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Importance of oxidative processes induced in normal and tumoral cell monolayers exposed to the action of cold plasma jets

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Résumé

We present the results of a study regarding the effects of atmospheric pressure cold plasma jets on normal (V79-4) and tumoral (HeLa) cell lines. Predominance of apoptotic or necrotic processes is described in relation to plasma contents and cell treatment times for two experimental setups: direct cell exposure and indirect diffused treatment.

Introduction

Atmospheric pressure non-thermal plasmas are nowadays extensively studied for their potential biomedical applications: treatment of certain types of skin cancers and dermatologic infections, burns, ulcers, blood coagulation inducement during surgery, sterilization of medical instrumentation, bacterial decontamination and others [1].

The conceptual possibility to adjust plasma and treatment parameters to ensure its non-invasivity, effectiveness and (ideally) selectivity regarding its action on different types of living organisms and cells has become a subject of interest and active research during the last several years [2]. Cold plasmas may possess chemical oxidative activity exposing biological material to oxidation processes mediated by the presence of reactive oxygen species (ROS) such as: superoxide anion, hydrogen peroxide, hydroxyl radical and singlet oxygen. These oxygen radicals are generated either within the plasma itself or as a consequence of the interaction between the plasma and the surrounding air. Based on our previous experience regarding the inducement of cell apoptosis under the action of chemically activated cold plasma jets (CPJ) [3,4], we investigate the presence of apoptotic and necrotic effects within cell populations exposed to a helium-oxygen non-thermal plasma. The experimental parameters that we have independently varied during the present study are the oxygen content of the plasma and the cell exposure times to the CPJ.

Experiment

The cold plasma jets used in our experiments was generated using pulsed high voltages with amplitudes in the range 20 – 30 kV, durations of 100 – 500 ns at half-maximum and frequencies of tens to hundreds of pulses per second. Plasma contained ROS derived from the molecular oxygen found in the initial composition of the helium-oxygen gas mixture.

We have used two cell lines: normal V79-4 (lung fibroblasts from Chinese hamster) cells and the tumoral line HeLa (human cervix cancer). The cells have been cultured with a number density of $1x10^6$ cells/ml in DMEM-F12 culture medium and allowed to become confluent. The plasma has been afterwards applied on cells in two experimental situations: directly, without the presence of the culture medium and indirectly by diffusion in the medium. For each case, the exposure times varied within the range 30-150s with a timestep of 30s. The helium-oxygen gas mixtures had the compositions He: 2.5l/min + O_2: X ml/min, the amount X of oxygen (O_2 ml/min) taking the values X=12.5, 25, 37.5 corresponding to 0.5%, 1%, 1.5% percents of oxygen in the plasma contents. We have determined the cell viability by means of the MTT technique and the ADP/ATP ratio using a dedicated commercial kit.

Results and discussions

In data analysis and interpretation we have considered the following aspects: a significant decrease of cell viability within the first two hours after the plasma treatment indicates the presence of necrosis. A relative reduced change in viability with respect to control within the first two hours following the exposure to the plasma jet reveals a small percentage of necrotic cells. Cell death detected afterwards is

attributed to apoptosis which, being an induced cell mechanism, implies a period of latency necessary for the specific cell modifications to take place.

Taking into account that the cell viability does not vary significantly, an increased ADP/ATP ratio is atributed to the inducement of apoptosis in a larger number of cells instead of necrosis which would affect a smaller number of cells producing a violent death and leading to the release of an ADP amount similar to that produced in the first case.

Since the ROS density within the cell environment depends on the oxygen percentage in the plasma gas mixture and on the treatment duration, we are justified to assume that the effect on the cell viability follows as a consequence of the action of the ROS produced within or by the plasma jet.

For the V79-4 cells necrosis was induced for all three helium-oxygen combinations used in the first experimental setup (direct action on cells). When culture medium was present (second experimental setup), the ROS had a more homogeneous action on the cells due to their diffusion in the medium before reaching the cell monolayer. In this case the apoptotic mechanism was induced for an oxygen amount of 12.5ml/min and necrosis was predominant for the other two cases.

HeLa cells exposed to plasma with an oxygen content of 12.5 ml/min became necrotic after 150s and 120s of treatment while for the other values of the treatment time (30, 60, 90s) an extended apoptosis was observed. Regarding the other two plasma compositions (O_2: 25ml/min and O_2: 37.5ml/min), based on the correlation of the ADP/ATP ratio and viability data, an extended apoptosis was diagnosticated for all plasma exposure times used in our experiments.

The ideal clinical use of atmospheric pressure plasma jets, in tumor treatment, would require the fine tuning of the device parameters aiming ensure a more aggressive exposure (leading to necrosis) inside the tumoral tissue and a less invasive treatment at the edges of the tumor in order to avoid the destruction of the adjacent healthy cells. Regarding these aspects, the plasma gas combinations He: 2.5l/min + O_2 12.5ml/min and He: 2.5l/min + O_2 25ml/min are of special interest. However, these ideas are difficult to implement in practice because of a poor delimitation between the tumoral and normal tissues.

A more realistic and safe alternative would be to use plasma jets at specific parameters in order to ensure a maximum percentage of apoptosis relative to that of necrosis. Our studies revealed such a situation for the He: 2.5l/min + O_2 37.5ml/min plasma gas contents, when apoptosis was induced for normal cells and, very important, for the tumoral cells too.

It is essential that the surface of the region about to be treated to be covered with a liquid/gel to ensure the diffusion of reactive species and the homogeneity of the treatment for the whole region of interest.

It is a matter of prime importance to establish a general procedure for finding those parameters, characteristic for each particular type of cell, for which a maximum percentage of apoptosis is obtained. In choosing those parameters one should take into account the types of cells involved in the considered disease and the fact that a reduced partial destruction of the normal cells at the treated site would be acceptable relative to the benefits brought in treating the cancer disease.

Acknowledgement

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Low-temperature microwave microplasma for bio-sterilization

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Résumé

We present results of the investigations of an atmospheric pressure argon microwave (2.45 GHz) microplasma which can be used in the biomedical applications. The microplasma in the form of a column was generated using a simple coaxial microwave microplasma source (MMS). The performance of MMS was tested at argon flow rate from 1 to 10 l/min and absorbed microwave power from 5 to 50 W. The length and diameter of microplasma ranged from 0.5 - 25 mm and 0.5 - 2 mm, respectively, depending on the operating parameters. The microwave power reflection coefficient (P_R/P_I) in the MMS was about 5% without any tuning elements. The spectroscopic investigations of the microwave microplasma were carried out to determine the electron density and the rotational temperature of heavy spices in the microplasma. The measured electron density varied from 10^{14} to 10^{15} cm^{-3}, depending on operating parameters and location within the microplasma column. The rotational temperatures were determined to be about 700 K for OH radicals and 800 K for N_2 molecules which were present in the microplasma due to the absorption of gases, including water vapour, from the ambient air. The gas temperature at the microplasma tip was as low as about 300 K. This makes the microwave microplasma suitable for many applications, also biomedical.

Introduction

The interest in the atmospheric pressure low-temperature microplasmas is growing because of many merits of such a microplasma: small size (from μm to several mm), portability of the source, easy to use, low investment and operation costs. The microplasmas can be used for gas cleaning, in microwelding and surface modification, as light sources, and atomic spectroscopy systems. Also there is interest in using the microplasmas in the biomedical applications such as sterilization of medical instruments, high-precision surgery, cells treatment and deactivation of bacteria and viruses [1-4]. Here we report results of the experimental investigation of a simple coaxial MMS [5] operated in argon. The presented MMS is a more advanced version of previous MMSs developed by us and described in [6-9]. The main advantages of the presented MMS are simplicity and low cost.

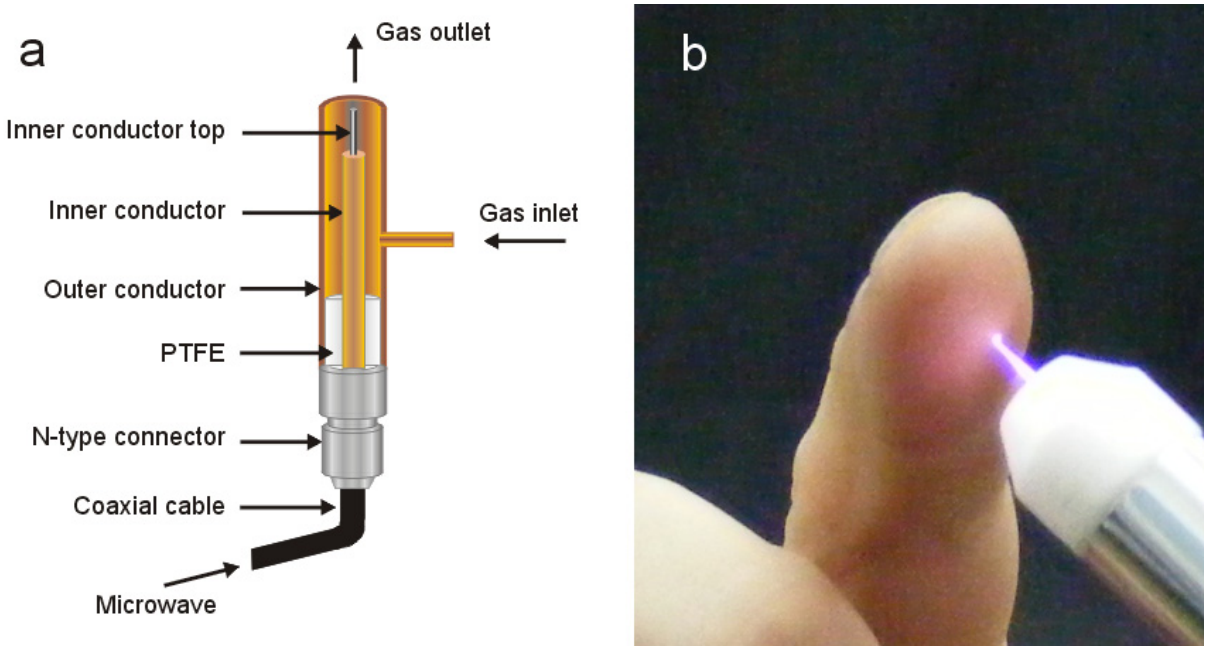

Fig. 1: The sketch of the MMS (a) and photo of low-temperature argon microwave microplasma (b).

The structure of the MMS is based on a coaxial line, formed by the inner (a brass rod ended with a thinner rod top) and outer (a brass cylinder) conductors (Fig. 1a). The top of inner conductor can be made of various materials (e.g. tungsten or graphite). The inner conductor is fixed inside the outer conductor tightly with a PTFE centering disc. The operating gas (in this case argon) was supplied through a void duct between the inner and outer conductors. The MMS was connected to the coaxial cable using N-type connector. The microwave power was supplied through a 50 Ω coaxial cable from a 2.45 GHz microwave magnetron generator. The argon microplasma generated by the MMS had the form of a tiny candle-like flame above the inner conductor top. Optionally the MMS could be operated with a PTFE tip, as seen in Fig. 1b. This tip played three functions: it formed a kind of nozzle that increased the velocity of argon in plasma forming zone, it prevented breakdowns between the inner and outer conductors, and it covered the hotter part of the microplasma column, thus exposing only the lowest temperature microplasma (i.e. its tip). The MMS was not equipped with any tuning element.

Experiments

To assess the usefulness of the argon microwave plasma for the biomedical applications, e.g. for sterilization, we performed spectroscopic measurements (Optical Emission Spectroscopy) of the electron density and microplasma temperatures. The main parts of the experimental setup used in these measurements were the magnetron generator (2.45 GHz), microwave power measuring system, the MMS, gas supplying and flow control system, and spectrometer (CVI DK-480 with 1200 gr/mm and 3600 gr/mm grating), equipped with a CCD camera and a PC computer, for emission spectra analysis. The microwave power P_{abs} absorbed by the microplasma was determined from the difference (P_I - P_R), where P_I and P_R are the incident and reflected microwave powers, respectively. The incident P_I and reflected P_R microwave powers were directly measured using directional coupler and dual-channel power meter. The measured power reflection coefficient (P_R/P_I) was about 5%.

The electron density in the argon microplasma was determined from the Stark broadening of H_β spectral line of the hydrogen Balmer series which was observed in the emission spectrum due to the presence of water vapour in the microplasma (from the ambient air). The rotational spectra of OH radicals ($A^2\Sigma^+ \rightarrow X^2\Pi$) and N_2 molecules second positive system ($C^3\Pi \rightarrow B^3\Pi$) were employed for the determination of rotational temperatures of OH and N_2 species. The measured spectra were compared with those simulated in SPECAIR program [10] in order to determine rotational temperatures of OH radicals and N_2 molecules. The rotational temperatures were determined to be about 700 K for OH radicals and 800 K for N_2 molecules at the microplasma column base. Using a thermocouple we found that the microplasma gas temperature at the microplasma tip could be as low as 300 K. These values were measured at an absorbed microwave power of 10 W. The obtained allows us to estimate the microplasma gas temperature.

Conclusions

The simplicity of the source, stability of the microplasma and wide range of its parameters allow the conclusion that the MMS can find practical applications in various fields. The usefulness the argon microplasma described in this paper for bio-sterilization is under tests.

Acknowledgement

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Time and space evolution of plasma bullets in APPJ applied for human tissue treatment

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Résumé

Plasma Medicine is a new interdisciplinary field developed in the last years that brings into biology and medicine new techniques from the plasma community. Many directions that involve plasma physics and biotechnology are in study nowadays in numerous laboratories. In this paper we present results related to the optical and spectroscopic diagnosis of a helium atmospheric pressure plasma jet, designed for medical applications, especially human tissue treatment.

Introduction

The increasing amount of reports and scientific publications related to the Plasma Medicine and its benefice / risks reflect the importance of plasma technologies use in fields like biology and medicine [1]. Atmospheric pressure jet discharges are known in literature under many names, such as: plasma pencil, plasma needle and atmospheric pressure plasma jet (APPJ). The variety of the atmospheric pressure plasma jet can be classified using different parameters such as: working gas, repetition frequency of the applied voltage pulse and the electrodes configuration. These parameters can be used in order to start a standardisation of the APPJ for bio-medical day-to-day use. In this study, an APPJ in helium with impurities was generated in a cylindrical dielectric barrier discharge (DBD) configuration and was characterized using spatio – temporal resolved optical emission spectroscopy and ultrafast photography.

APPJ set-up

The experimental arrangement of the plasma jet designed in our laboratory is shown in fig. 1. We used a quartz tube (inner diameter 4 mm, outer diameter 6 mm) with one copper slit electrode. High voltage pulses, 2 kHz frequency, 260 μs width and 4 kV amplitude, are applied from an amplification chain: a high voltage amplifier (Trek Inc.), driven by a square pulse waveform generator (Tabor Electronics Ltd.). Helium (spectral purity) was used as working gas for the APPJ, at a flow rate of 3 slm (2.5 m/s, calculated speed) [2].

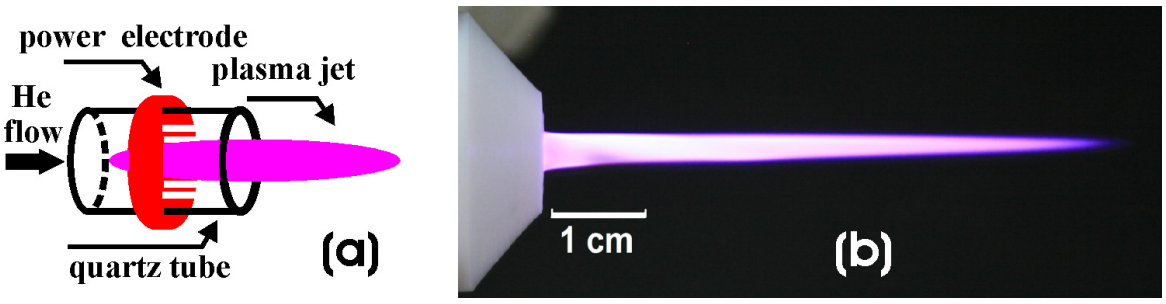

Fig. 1: (a) Scheme and (b) photo of our APPJ.

The discharge properties were analyzed by optical emission spectroscopy and ultra fast imaging. The light emission from the DBD plasma jet was collected through a slit with an optical fibre placed at 5 mm from the plasma jet, and then analyzed with a high resolution Triax 550 (Horiba Jobin Yvon) spectrometer equipped with a Symphony CCD (Horiba Jobin Yvon) and a Hamamatsu photomultiplier as detectors. The optical emission spectra were collected in different positions of the jet's principal axis in order to identify and to monitor the plasma active species. The rotational and the vibrational temperature of excited molecules in the plasma volume were calculated from the high resolution spectra of nitrogen molecular ion (N_2^+) at 391 nm and the second positive system of molecular nitrogen (N_2). An ICCD

camera (Hamamatsu C8484-05G) was used to capture 50 ns exposure time photos of the plasma jet, for a better understanding of its dynamics.

Spatial resolved optical emission spectroscopy and ultra fast imaging

The APPJ spectrum contains lines from helium transitions, the plasma working gas, and signatures of impurities, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), as shown in fig 2(a). More precisely the optical emission spectrum of the APPJ consist of the following atomic lines / molecular bands: OH radical – at 308.9 nm as a result of H_2O dissociation, N_2 – at 337 nm as dominant component of the ambient air, N_2^+ - at 391 nm, an helium metastable presence indicator, He – at 706 nm as plasma working gas, O – at 777 nm and 845 nm as a result of H_2O dissociation as well. The axial dependence of these lines or bands is analysed and discussed. From the rotational and vibrational spectra of N_2^+ and N_2 we determined the rotational temperature, $T_{rot} \approx 470$ K, and vibrational temperature, T_{vib} from 3500 to 2500 K.

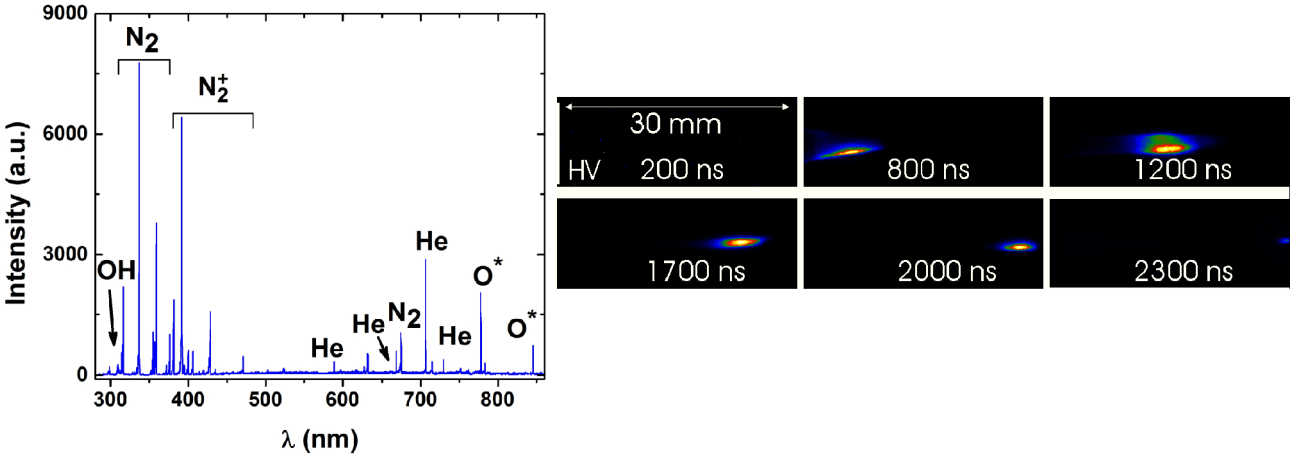

Fig. 2: The APPJ (a) emission spectrum and (b) 50 ns exposure ICCD images

The ultra fast imaging investigations (Fig. 2(b)) show that our APPJ has a bullet-like behaviour. The propagation mechanism of this type of discharge can be describe either by an ionization model [3-4] or a streamer model [5]. The high speed plasma structures reach velocities, calculated from ICCD images, ranging between 0.1 to 14×10^4 m/s outside the discharge tube. The plasma bullets velocities can be determined also using a photomultipliers (fast response time).

Conclusion and perspectives

The APPJ in helium, in our experiments, have many reactive species (N_2, N_2^+, OH, O^*), their evolution, in time and space being important for medical application. The rotational, as well as the vibrational, temperature was determined to be around 470 K, respectively between 3500 – 2600 K.

Using an ultra fast camera (ICCD) plasma bullets velocities were determined to range between 0.1 to 14×10^4 m/s. This peculiar spatio – temporal behaviour of the APPJ must be correlated with its biological effects (e.g. healing), since tissues or biomolecules exposed to this kind of plasma jet are irradiated with temporal and spatial noncontinuous plasma structures.

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Ozone soil conditioning and decontamination

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Résumé

This work introduces an overview on the bactericidal properties of plasma assisted ozone treatment for the soil remediation. Developed electrode system provided ozone concentration beyond 20 g/m^3, which is the critical value of soil sterilization. The gaseous ozone injection system consisted of 10 injectors and the treatment container, which was developed to sterilize a large volume of agricultural soil. A stream of oxygen containing 5% by weight of ozone was bubbled into the microcentrifuge tube containing the λ-E.Coli DNA solution. 0.2-1 gram of ozone was supplied to 0.5 milliliter of DNA solution during 5-20 min of treatment with 0.5 liter/min causing complete collapse of the DNA structure.

Introduction

Sterilizating techniques employing active species such as ozone, hydrogen peroxide, OH radicals, oxygen singlets are especially beneficial for persistent microbial pollutions caused by the aggregations of microorganisms called biofilms, which are widely present on various surfaces and within soil. Anti-microbial properties of plasmas in the case of decontamination of water, ambient air and surfaces were previously widely proven [1-4]. Pollutants might be distributed in soil in several ways: in soil matrix, vapor phase, non-aqueous phase or groundwater [5]. Ozone based techniques are good alternative to traditional techniques like heating, flushing with chemical additives, landfilling, incineration, etc. Benefits of ozone applications in agriculture might be summarized as follows: use of ozone in soil treatment will not result in the build-up of any environmentally persistent or toxic compounds but ozone itself, and O_3 is immediately consumed in the soil treatment process; ozone is manufactured on site so it cannot be stored and its sudden release into the atmosphere is not possible like it could occur with compressed methyl bromide or other persistent, toxic gases or chemicals used for soil sterilization; thus, it assures minimum human severe and toxicity.

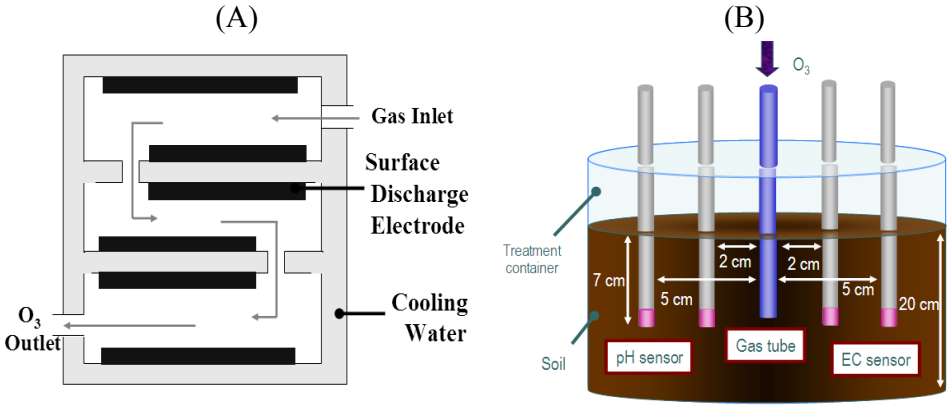

Fig. 1: OP-20W ozonizer (A), Multi-electrode injection system (B).

Experimental set-up

The main element of soil sterilization system was TiO_2 based surface discharge commercial OP-20W Iwasaki ozonizer, which is presented in Fig. 1(A). The gaseous ozone injection field-scale system is shown in Fig. 1(B). It consisted of 10 injectors and the treatment container, which was developed for sterilizing and monitoring of agricultural soil in large volume. The pH value, electrical conductivity and temperature of the soil were observed to investigate the effect of ozone treatment on soil properties.

Results and discussion

Conventional biological method of the CFU (colony forming unit) counting showed that bacteria and Fusarium oxysporum in the soil were almost eliminated by ozone treatment with the concentration over 20 gO_3/m^3 achieving sterilization rate up to 99.9%. The dependence of sterilization rate on the amount of ozone introduced to the soil is presented in Fig. 2(A).

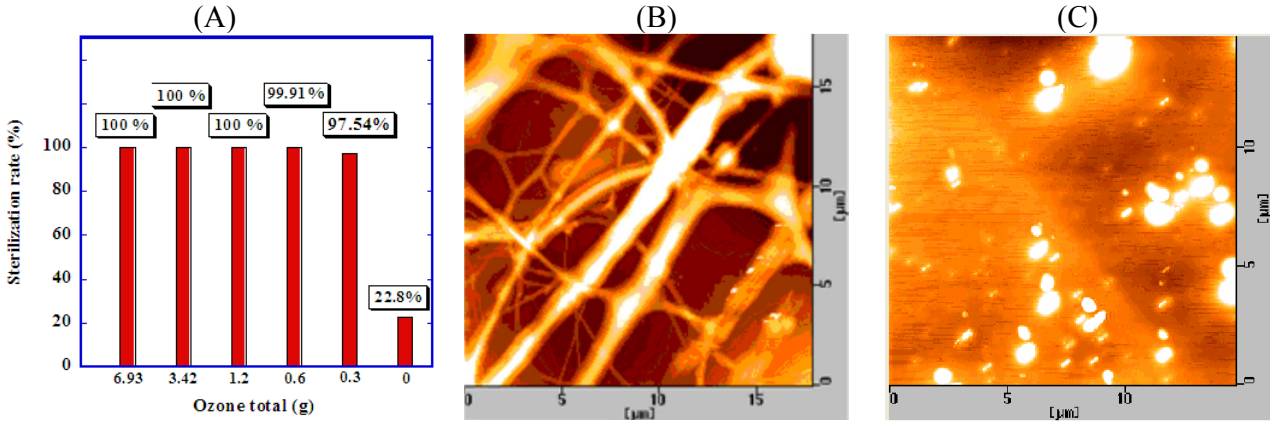

Fig. 2: Sterilization rate vs. ozone concentration (A), Sample of DNA E.coli (AFM, 48,502 base pair,16µm) before ozonation (B), Ozone treated DNA (C).

λ-E.Coli DNA (Nippon Gene) was diluted with Tris-HCL and EDTA. The DNA solution was further diluted with distilled water in a microcentrifuge tube. A stream of oxygen containing 5% wt. ozone was bubbled into the microcentrifuge tube containing the DNA solution. 0.2-1 g of O_3 and was supplied to 0.5 ml of DNA solution during 5-20 min of treatment at 0.5 l/min gas flow rate. Fig. 2(B) depicts the image of DNA sample deposited on the mica substrate. The molecular structure of DNA collapsed completely when high concentration of ozone was introduced into the DNA solution. Treated DNA sample is presented in Fig. 2(C). It indicated that ozonation process broke the E. coli DNA and split it into many fragments of typical length and width of 380-390 nm and 15 nm, respectively. These peculiar pieces have almost been not observed when the DNA samples were treated for longer time, when DNA was decomposed completely.

Conclusions

The set up for agricultural microbial ozone sterilization purposes was developed. Gaseous ozone sterilization was proven to be satisfactory for treatment of soil infected by Fusarium oxysporum. Up to 99.9% sterilization efficiency was achieved using ozone dosage over 20 gO_3/m^3.

The fundamental experiments on biological reaction between the λ-E.Coli DNA and ozone exposure suggested that the molecular structure of the DNA collapsed completely using 5% wt. ozone concentration.

The ozone can be successfully used for the soil remediation and removal of biological agents present in plasmonic and biofilm forms.

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Comparison of direct and indirect effects of cold air plasma on bacteria contaminated surfaces

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Résumé
Experimental work concerning non-thermal air plasma treatment of gram-negative bacteria *Salmonella typhimurium* on agar surfaces is presented here comparing direct and indirect exposition to the plasma for various times of exposition. The results are characterized by visible differences of inactivated areas between these two methods of treatment.

Introduction

Bacteria inactivation with non-thermal atmospheric pressure plasma in air is a highly complex process including many possible stress agents, such as charged particles, reactive neutral species, UV and electromagnetic radiation and heat. In recent years, the most discussed agents mainly contributing to efficiency of plasma induced bio-decontamination are charged particles and reactive neutral species [1, 2, 3].

Experiment

In our work, bacteria contaminated agar surfaces are treated by non-thermal atmospheric pressure air plasma induced by DC driven electrical discharges. Experimental setup was prepared the same way as in [4] to compare direct and indirect exposition of the agar surface on Petri dishes to the discharge. This was especially set to separate reactive neutral species and charged particles from the plasma.

Positive transient spark (TS), a DC-driven electrical discharge used in point to-plane geometry, is a transient streamer to spark discharge with self-driven pulse regime. TS is characterized by relatively high energy in very short pulses (10 – 100 ns) with temperatures ~ 550 ± 100 K [5]. The current pulses were of amplitude 2 – 3 A, with frequencies around 1 – 2 kHz and the average power of ~ 2 W.

Our biological sample *Salmonella typhimurium* was prepared by cultivation in liquid nutrient broth and then spread onto a solid agar over the whole surface in Petri dishes. Prepared samples were directly or indirectly treated with plasma and incubated for 12 h. Exposure time was set to 5, 10, 15 s, which is much shorter than in our previous work [4]. In the Petri dishes for direct exposition, a conductive wire was immersed into agar, acting agar surface as one electrode. In indirect exposition, we placed a grounded mesh over the agar surface. This mesh filters the charged species and enables only neutral active species to reach the agar surface. Schematics of the experimental set-ups is in figure 1. In both exposition methods we tried to ensure same electrical parameters. For this purpose, additional resistor simulating agar resistance was added between the mesh and the ground. The experiments were repeated 3 times with initial bacterial populations on agar surface of 10^4-10^5 CFU.

Results

The effects of plasma on contaminated agar are clearly visible as dark voids (Figure 2), whereas control samples were homogeneously covered by cultivated bacteria (bright). The results show that direct exposition has slightly stronger effect than indirect. However, even for small times of exposure, the effect of the indirect exposition is comparable with the direct exposition. Similar results were obtained previously for 1-2 min exposure times. This indicates that neutral reactive species generated in the discharge are crucial in bacterial inactivation even at very short times (5-15 s).

Acknowledgements

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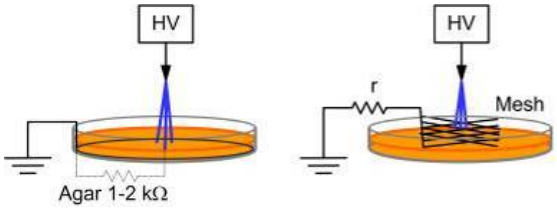

Fig. 1: Schematics of experimental setup, left: direct; right: indirect exposition.

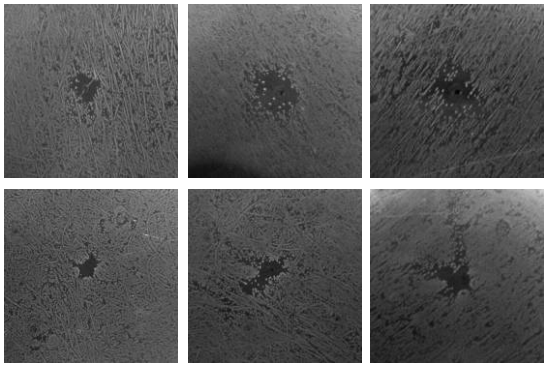

Fig. 2: Plasma treated agar surfaces with *Salmonella typhymurium* for various exposition times, top: direct, bottom: indirect exposition.

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DBD plasma assisted silver functionalization of surgical meshes

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Résumé

Atmospheric pressure diffuse barrier discharge in nitrogen was employed to promote the formation silver sub-micron particles on PP and PES warp-knitted surgical meshes. Its good antimicrobial efficacy against gram- positive *Stafylococcus aureus* and gram-negative *Escherichia coli* was confirmed.

Introduction

Owing to the substantial reduction of recurrence rate, the use of surgical meshes has become a standard procedure in hernia repair surgery throughout the world during last decade. At the same time however the mesh related problems, such as mesh-related infections, has risen into importance. One of approaches to prevent the mesh infection is to embed antimicrobial agents into the mesh, which prevent bacterial adhesion and further colonization when implanted into wound [1].

In our work we have attempted to prepare silver particles functionalized meshes. Silver ions are well recognized for their high toxicity for microorganisms while being is the least toxic metallic ion to animal cells [2]. To initiate the formation of cluster of silver atoms in solvent such as water, silver ion has to be first reduced down to the zero-valent state [3]. In our experiment radicals created by N_2 atmospheric pressure plasma treatment of mesh surface were employed as the reducing agents. The uniformity of created surface radicals was accomplished by generating plasma by diffuse mode of dielectric barrier discharge. It was found that the growth of silver atom clusters occurs preferentially on surface of surgical meshes yarns.

Surgical warp-knitted meshes made of PP monofilaments yarn and PES filaments were treated by 20 kHz, 100 Watt N_2 diffuse dielectric barrier discharge (APGD) for various treatment times. Immediately after the treatment, mesh samples were immersed into water solution of $AgNO_3$ of various concentrations and temperature. Quantitative analysis of immobilized silver was done by the potentiometric titration. Morphology of silver particles was studied by SEM. The antimicrobial efficacy of samples was evaluated in accordance with ISO 20645:2004, Textile fabrics - Determination of antibacterial activity - Agar diffusion plate test. The results showed that 2 min exposure to N_2 diffuse DBD, followed by 60 min dip in 0.05M $AgNO_3$ is sufficient to secure good antimicrobial efficacy against gram- positive *Stafylococcus aureus* and gram-negative *Escherichia coli*, with the inhibition zone greater than 1 mm (so called good effect). The reference samples without the treatment did not exhibit any antimicrobial activity.

Acknowledgement:

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Comparison of various types and parameters of corona discharges for decontamination of surfaces and liquids

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Résumé

We studied the decontamination of surfaces and water suspension of microorganisms by low temperature plasma generated in the DC corona discharge of an open-air type in the point-to-plane or point-to-point arrangement. We found, that different types of inhibition zones appeared on surfaces, which indicates the different mechanism of action of the point-to-point and point-to-plane dicharges. In liquids, the efficiency of decontamination is more or less equal for various bacteria and much lower for the eukaryotic yeast.

Introduction

The action of the plasma generated by electric discharges is one of the possible methods of inactivation of bacteria and other microbes, mediated by the bactericidal action of UV light and reactive particles. The various experimental arrangements, advantages and status of research in this field were reviewed in details in many works, e.g., [1], [2], [3], [4] and [5]. This contribution is a part of our systematic study of properties and differences of various types and arrangements of DC discharges and their microbicial effects.

Apparatus, methods and the microorganisms under study

We studied the inactivation of microbial suspensions in wells of a dot plate or on wet surfaces by low temperature plasma generated in the DC corona discharge. The used simple apparatus of an open-air type enabling the point-to-plane or point-to-point arrangement was previously described in [6]. The point-to-plane corona discharge was generated on the point electrode represented by the tip of a hypodermic needle and situated 4 mm over the sample. The plane anode was either the suspension in the well or the conducting surface of an agar cultivation medium. The bipolar point-to-point corona discharge was generated on a pair of hypodermic needles arranged in an angle of 30° with tips approx. 4-6 mm apart and situated 4 mm over the sample.

For the decontamination of liquids, 0.5 ml of the suspension of microorganisms was pipetted into the sterile wells of a dot plate, grounded and exposed to the discharge. Following the exposition, the content of each well was diluted, spread onto the surface of cultivation medium and after the overnight cultivation at 37 °C, the numbers of survival colonies were counted.

For the surface decontamination, 1 ml of the microbial suspensions were inoculated onto the surface of Sabouraud or Mueller-Hinton agar (Lab M, Ltd.) dilluted to obtain the microbial concetration of 10^6 cfu cm^{-2}. After the suspension soaked, the samples were exposed to the corona discharge, incubated at 37 °C for 24 hours and the inhibition zones were measured.

All expositions were performed under laminar flow of HEPA-filtered air to prevent the airborne contamination, the ambient conditions were controlled by an air-conditioning of the laboratory.

The microorganisms under study were "wild" strains of the following species isolated at the Institute of Immunology and Microbiology: a yeast *Candida albicans*, Gram-negative bacterium *Escherichia coli* and Gram-positive bacterium *Staphylococcus epidermidis*.

Experiments

In the case of decontamination of liquids, the parameters of discharges were adjusted as follows: for the positive transient spark corona to U = 10 kV, 8 kV or 6 kV and I = 200 μA; for the negative pulseless glow regime to U = 9 kV and I = 180 μA; for the negative glow discharge to U = 9 kV and I =

370 μA. Using the different parameters respects the different character of particular discharges. The samples were exposed up to 8 min.

In the case of decontamination of surfaces, the point-to-plane discharge was adjusted to the current I = 50 μA, its voltage was U = 4.6 kV. The point-to-point corona discharge was adjusted to the current I = 200 μA and voltage U = 10 kV. The different parameters for particular discharge types are determined by the different character and geometry of discharges. The values represent a compromise between discharge stability (transition into spark) and its energy and enable the qualitative comparison of all discharge types. The samples were exposed for 8 minutes.

Results and discussion

In liquids, the total inactivation of bacteria becomes after 60 seconds of exposition to the positive discharge and after 75 seconds of exposition to the negative one. On the other hand, exposition times of 8 or 10 minutes are necessary for the total inactivation of yeast by positive or negative discharge, respectively. The best efficiency of decontamination was found for the positive transient spark corona discharge at voltage in the interval of U = 8 – 10 kV and current of I = 200 μA. The negative glow discharge is equally or more effective than the pulseless one. The inactivation of the eukaryotic yeast is less effective and requires 8 - 10 times longer expositions.

Interesting results were obtained after exposition of inoculated agar surfaces. The point-to-plane discharge produced circular and sharply bordered inhibition zones with diameters of 5 - 6 mm, which were completely clear and contained no growing microbes. In contrast to liquids, the greater zones and thus greater sensitivity to the discharge were observed for *Candida* than for bacteria. After exposition to point-to-point discharge, we obtained two types of asymmetric fan-shaped zones of incomplete inhibition. In the case of *Candida albicans* yeast and Gram-positive bacterium *Staphylococcus epidermidis*, the inhibition zones were well bordered and almost clear of any surviving colonies, whereas for the Gram-negative bacterium *Escherichia coli* we obtained larger fan-shaped zones containing the reduced number of surviving colonies and very small, if any, zone of total inhibition only. The areas of total inhibition were approximately of S = 3 cm^2 for *Candida albicans* and S = 1 cm^2 for *Staphylococcus epidermidis*, the zone of incomplete *Escherichia coli* inhibition was of S = 10 cm^2. The different plasma-chemical processes in the discharges and the different reaction mechanisms with the microbial cell wall are probably responsible for these effects.

Conclusions

It can be concluded that the positive transient spark corona dispays the best efficiency of liquids decontamination. The efficiencies of used discharges are very similar, so that the precise control of their parameters is not necessary in the possible practice.

The results of decontamination of surfaces imply the different mechanisms of microbial inactivation by the point-to-point and point-to-plane discharges. Whereas point-to-plain discharge produces uniform and nearly circular inhibition zones of total inhibition, the character and appearance of two zone types produced by the point-to-point discharge was different. This fact supports the assumption that the sterilization agents are of different nature in point-to-point and point-to-plane discharges, displaying different efficiency and probably different mechanisms of action on microbial structures.

Acknowledgement

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Bio-decontamination of plastic and dental surfaces with atmospheric pressure air DC discharges

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Résumé

In our experiments we have been exploring bio-decontamination effects of atmospheric pressure air discharges applied on plastic and dental surfaces contaminated by bacterial spores (*Bacillus cereus*). We found discharge regimes with good bactericidal effects and low energy requirements.

Introduction

Prions and numerous bacterial strains become resistant against commonly used chemicals disinfectants and antibiotics. Conventionally used techniques (autoclave and dry heat) cannot by applied for human tissues or some plastic materials, such as polypropylene and polyethylene. Therefore, searching for new methods in decontamination of heat-sensitive materials is crucial in medicine and food processing. Cold air plasmas at atmospheric pressure provide an alternative with great potential, because there are efficient and do not cause degradation of termo-sensitive materials [1,2]. Cold plasmas are capable to disinfect dental plaque and caries and so could become a good alternative to painful teeth drilling [3].

We used non-equilibrium air plasma at atmospheric pressure produced by DC discharges for bio-decontamination of plastic and dental surfaces with bacterial spores of *Bacillus cereus.* We treated spores on a smooth surface of polypropylene foil or on rough dental surfaces and in dental cavities of extracted human teeth. We tested the impact of corona discharge in both polarities and the effect of moisture. A detailed description of our discharges used can be found in [4,5].

Our discharge set-up contains hypodermic injection needle as high voltage (HV) electrode and a grounded copper plate. The gap between the HV electrode and the sample was 0.5 cm for both polarities. We placed circular plastic foils (2 cm diameter) or a tooth with 20 µl of spore suspension droped on the surface of the plate electrode. A spore suspension contained around 10^6-10^7 colony forming units per ml.

At first we made experiments with polypropylene plastic foils under moist or dry conditions, with both polarities of corona. Each discharge regime was applied to at least 4 samples in 5 experimental series, and each sample was exposed to the discharge for 2 minutes. Positive corona was supplied with a HV ~11 kV, this formed streamers (current pulses) with frequency 7-12 kHz and maximum amplitude 30 mA. Negative pulsed corona was supplied with 8-9 kV, its amplitude was ~0.3 mA and frequency 1 MHz. When HV was further increased, negative corona established a pulseless regime with constant current 0.22 mA and constant voltage 9.4 kV. Under physiological conditions teeth surfaces are wet; therefore we treated the teeth as the moist plastic samples. So far we did 3 experiments with corona on 8 dental samples. Teeth were exposed to the plasma for 3 or 5 minutes.

Figure 1 shows the results of bio-decontamination of the spores on plastic and teeth surfaces in efficiency vs. energy graphs. On dry plastic samples, positive streamer corona is more effective and more energetic, whereas negative pulse corona is more efficient on moist ones with about the same energy consumption. Negative pulseless corona is largely more energetic and so it is not so convenient for bio-decontamination, despite it provided the best sporicidal efficiency. From the first few experiments with moist teeth surfaces we can see that the efficiency in 3 and 5 minutes exposure time is almost the same. Positive streamer corona was the most efficient and negative Trichel pulse corona slightly less efficient and less energetic.

Figure 2 shows the photographs taken by Olympus E410 camera with long exposure times. Each picture was made from the side of the discharge. The photos show discharges on plastic and teeth samples placed on the plate electrode and the needle (electrode above).

Our results from decontamination of bacterial spores on both plastic and dental surfaces are satisfying, taking into account that spores are extremely resistant to adverse conditions. The next step

will be treatment of dental surfaces contaminated by *Streptococcus mutans* biofilms in tooth cavities representing a dental plaque.

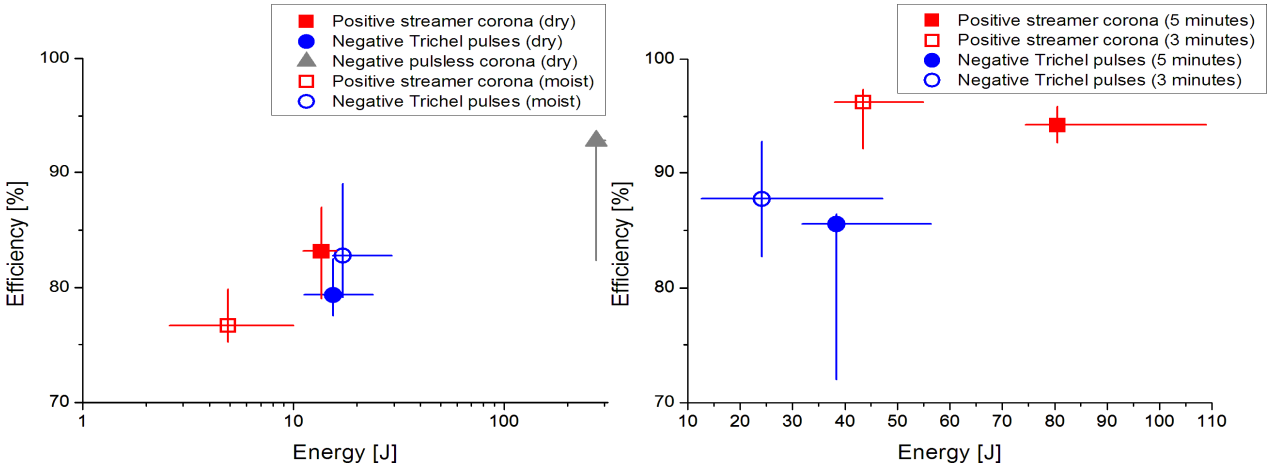

Fig. 1: Decontamination efficiency vs. energy (median with first and third quartile): plastic samples (left) and moist tooth samples (right)

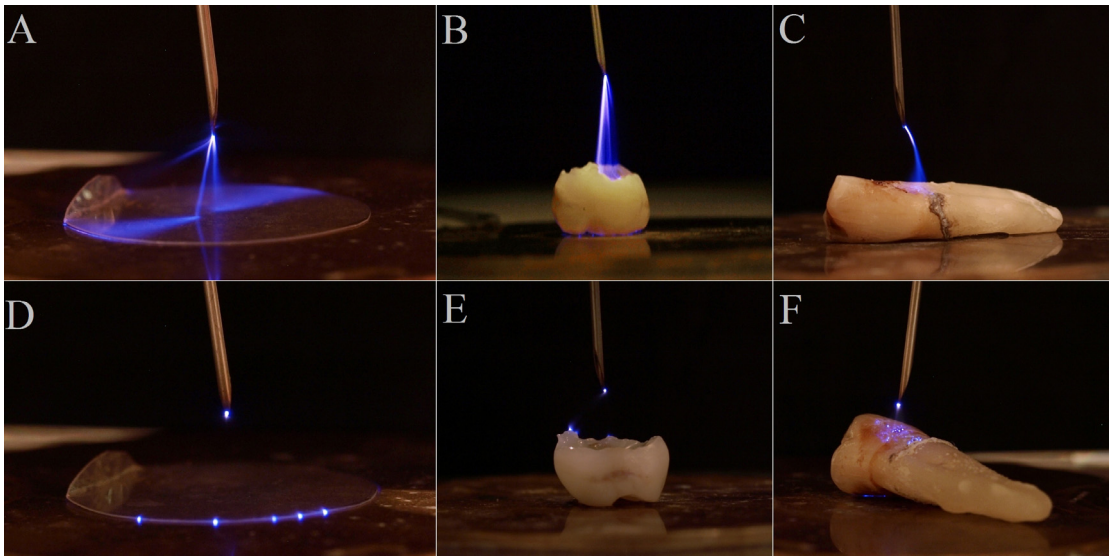

Fig. 2: Photographs of contaminated plastic (A, D) and tooth (B, C, E, F) samples with atmospheric pressure air DC discharges: 1st row (A, B, C) - positive stramer corona, 2nd row (D, E, F) - negative corona (Trichel pulses). (Exposure times [s]: A 5; B 1/2; C 2; D 3.2; E 1.3; F 2.5)

Acknowledgements

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The effect of ionizing gas plasma as apoptosis promoter in some cancer cell lines

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Résumé

The low-temperature atmospheric plasmas have provided a cheaper and more convenient alternative to low-pressure plasma technology.

In order to be used for biological materials, they must generate non-thermal plasmas (below 40ºC), should be operated at atmospheric pressure and should not cause electrical or chemical damage to the biological materials.

The incidence of melanoma has significantly increased in many parts of the world and it is one of the main causes of death and morbidity from cancer. Treatment of a melanoma has relied on chemotherapeutic agents, which induces apoptosis in cancer cells. However, once melanoma spread beyond the skin, it is frequently incurable by available chemotherapeutic agents. This is mainly due to the development of resistance to apoptosis.

In the present work human melanoma SkMel63 cell line shown to be killed immediately by high doses of plasma treatment, moreover, low doses shown to promote apoptotic behavior as detected by flow cytometry. It is shown that plasma acts on the cells directly and not by "poisoning" the solution surrounding the cells, even through a layer of such solution. Potential mechanisms of interaction of plasma with cells are discussed and further steps are proposed to develop an understanding of such systems.

Generation of reactive oxygen species in kHz-driven atmospheric pressure plasma jets for biomedical applications

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Résumé

We have developed a kHz-driven plasma jet for the generation of reactive oxygen species for biomedical applications. When operated in helium with small oxygen admixtures, our jet device produces stable non-equilibrium "plasma bullets" at atmospheric pressure. These small and fast plasma packets are emitted into ambient air, and, thus, able to carry several species into biological targets positioned some centimeters away. Optical diagnostics of the effluent region have been performed, allowing the measurement of singlet delta oxygen and ozone absolute densities. High concentrations of these reactive oxygen species (10^{14}–10^{16} cm^{-3}) have been obtained at 5–15 cm downstream. Moreover, it has been observed that the control of the jet operating conditions enables to tailor the reactive oxygen species composition of the effluent towards different biomedical applications, from fundamental biochemical studies to therapeutic treatments, through sterilization and bio-decontamination.

Introduction

Cold plasma jets produced by pulsed discharges have recently attracted a lot of attention because of their unusual physical properties that enable the development of new applications, particularly for biomedical applications. The current interest in this type of atmospheric pressure discharge stems from the fact that they provide a means of delivering, at ambient pressure and temperature, reactive plasma species/elements (radicals, positive or negative ions, electrons, UV radiation), and not only long-lived afterglow species, to a target located some centimeters away from the main discharge zone. In fact, the plasma is not spatially confined by the electrodes, and intensified charge coupled detector (ICCD) pictures of these jets revealed that they were not continuous plasmas but were composed of "comet like" discrete plasma pulses (commonly known as "plasma bullets") propagating at very high velocity, much greater than the discharge gas velocity [1]. These stable non-thermal plasmas can travel several centimeters in the ambient air to deliver reactive species to the surface of a biological sample.

Many different kinds of cold plasma jets have been developed [2–5]. The simplest type of these sources consists of a dielectric tube with two tubular metal electrodes and a noble gas flowing through (linear tube jet) [6]. As shown in Figure 1, the plasma jet design investigated in this work is composed of a capillary dielectric tube (quartz) with inner diameter of 4 mm and outer diameter of 6 mm. Tubular copper electrodes (2 mm wide) are assembled around the tube separated by a few centimeters from each other. The powered electrode is driven with a 20 kHz pulse repetition and high voltage (4–10 kV) supply. The electric field is directed parallel to the gas flow. Helium is used as the main discharge gas carrier with 1–6 slm flow rate. The plasma jet is operated in He/O_2 mixtures (O_2 <2%), and the effluent is emitted into ambient air. An intense plasma forms inside the glass tube between the two electrodes, and a relatively long pulsed plasma plume (few cms) emerges at the end of the capillary tube. The length of the plume has been found to depend on the operation parameters (e.g. applied voltage, gas flow rate).

With the increasing development and usage of plasma devices in the treatment of living tissue and the full effects of its application on DNA still unknown, much research still needs to be carried out in this area. This work intends to improve the understanding of how plasma treatment can affect DNA by correlating measured reactive oxygen densities in the effluent of a plasma jet to its influences on DNA. In our experimental setup, DNA solutions can be placed in wells and placed in front of the plasma effluent (cf. Figure 1). As it has been recently demonstrated [7,8], exposure to oxygen-containing atmospheric plasmas can result in DNA damage. In the present work, absolute densities of reactive oxygen species are measured by different optical methods: singlet delta oxygen (SDO) by infrared emission spectroscopy [9], and ozone (O_3) by ultraviolet absorption spectroscopy. These densities can be directly correlated with

DNA damage, which allows attribution of species to certain types of damage, and gives scope to tune the plasma for desired effects.

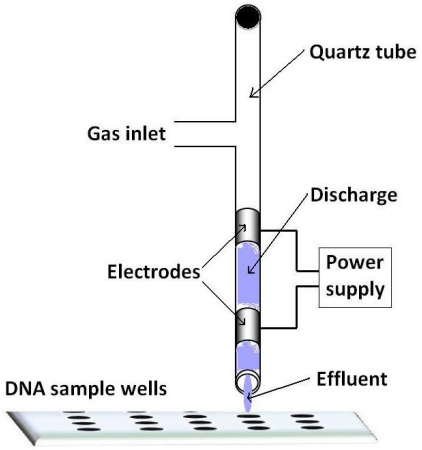

Fig. 1: Schematic of the kHz-driven atmospheric pressure plasma jet.

Results and discussion

The effect of different parameters, such as gas flows and mixtures, and power coupled to the plasmas, on the production of O_3 and SDO by the plasma jet has been studied. High concentrations of these reactive oxygen species (10^{14}–10^{16} cm^{-3}) have been obtained at 5–15 cm downstream. As exemplified in Figure 2, by controlling the discharge operating conditions, we are able to tailor the reactive oxygen species composition of the jet effluent. While at very low oxygen concentration in the gas mixture (~0.3%) similar densities are measured for O_3 and SDO, the density ratio of O_3 to SDO increases considerably with increasing oxygen admixture, up to 30 at about 1.5% of oxygen. In order to determine the relevance of these reactive oxygen species in DNA oxidation, studies of the interaction of DNA solutions with the jet effluent are currently in progress.

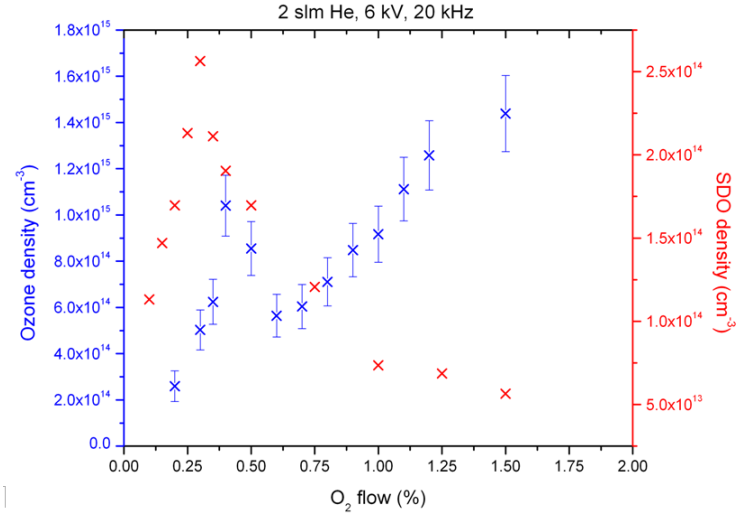

Fig. 2: Evolution of the SDO and the O_3 densities as a function of the oxygen fraction while operating the plasma jet at 6 kV and at 20 kHz, in a He/O_2 mixture, with a He flow of 2 slm.

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The fungal spores survival under the low-temperature plasma

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Résumé

Microbicidal effects of low-temperature plasma generated in electrical discharges are a well established area of research and the mainstream of study of several laboratories in the world. This paper presents an experimental apparatus for the decontamination and sterilization of water suspension of fungal spores. The fungicidal effect of stabilized positive and negative flashing corona discharges on three fungal species *Aspergillus oryzae, Clacosporium sphaerospermum* and *Penicillium crustosum* was studied. Simultaneously, the slower growing of exposed fungal spores was observed. The obtained results are substantially different in comparison with those of the analogous experiments performed with bacteria. It may be concluded that fungi are more resistant to the low-temperature plasma.

Introduction

There are numerous works describing the biological effects of low-temperature plasma generated in electrical discharges, devoted mainly to the killing of prokaryotic bacteria (see reviews Laroussi 2005, Scholtz et al. 2007, Moreau et al. 2008) or various applications in human medicine (Fridman et al. 2008). Concerning fungi, only Akishev et al. (2008) mentioned the inactivation of *Aspergillus niger* and *Candida lipolytica* on agar surface after exposure with the plasma jet device. The possible application of plasma sterilization may be useful e.g. for treatment of fruits´ surface, preventing the mould overgrow and bacterial putrefaction.

The low temperature plasma was generated using the previously described (Julák et al. 2006) simple point to plane apparatus of an open-air type. The ground electrode was realized by the surface of water suspension of microorganisms. All exposures were performed under laminar flow of HEPA-filtered air to prevent the airborne contamination; an air-conditioning of the laboratory controlled the ambient conditions. The suspensions of conidia were prepared immediately before the exposure to the positive and negative discharge and exposed for various time intervals. The effects of spores inactivation and dynamics of the micromycete growth after exposure were observed.

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Decontamination of biological suspensions by pulsed corona discharges: Role of UV radiation, frequency and conductivity

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Resumé

Decontamination of bacterial suspensions by a pulsed corona discharges generated in a liquid phase was investigated. The inactivation effect of the pulsed corona discharge was studied in dependence on the solution conductivity (200 and 500 μS/cm), on the type of microorganism and the pulse repetition frequency. The role of UV radiation emitted by the electrical discharge in the overall bacterial efficiency was evaluated in dependence on the solution conductivity using a UV light transparent spectrometric cell. The reactor with a point to plate geometry of electrodes was used for generation of the discharge in liquid. Bacterial suspension of *Escherichia coli* and *Enterococcus faecalis* were used. Exposure of living forms of *E. coli* and .*E. faecalis* were inactivated by the pulsed corona discharges with respect of increasing conductivity. It has been shown that the contribution of UV light during the pulsed corona discharges is very important.

Introduction

Previous research has demonstrated that the high voltage pulse electrical discharges generated directly in the liquid phase initiate a variety of chemical and physical processes. These processes include a high electric field, intense ultraviolet radiation, overpressure shock waves and formation of various highly reactive chemical species such as radicals, molecular radicals and ions. It was shown that these processes are capable to efficiently destroy a number of organic compounds and cause serious damages to microorganisms present in the liquid phase [1, 2].

Concerning the bactericidal effects of UV radiation, UV light (200–300 nm) with doses of several $mWs.cm^{-2}$ is known to cause lethal damage to cells. UV radiation affects the cells of bacteria by inducing the formation of thymine dimmers in the DNA. It suppresses replication of DNA [3, 4].

Electrical discharges in liquid phase can emit significant intensity of UV light [5]. Previous research obtained using the emission spectroscopy showed a radiation from the pulsed corona discharge in liquid phase in a wide range of wavelengths (200–1000 nm), which is dominated by the spectral lines of hydrogen (peaks at 434, 486, 656 nm) and oxygen atom (777 nm) and by emission from OH• radical (309 nm) [6, 7]. Consequently, Lukes et al. have determined that pulse radiant power (190–280 nm) of the corona discharge in liquid phase could reach levels of the order of tens to hundreds of watts during the pulse, which corresponds to the UV radiation intensity of the order 0.1–10 $mW.cm^{-2}$ in dependence on solution conductivity [5].

In this work the role of UV radiation in the bacterial inactivation caused by the pulsed corona discharge in the liquid phase is investigated in more detail. The inactivation effect of the pulsed corona discharge is studied in dependence on the solution conductivity (200 and 500 $\mu S.cm^{-1}$) and on the type of microorganism, *E. coli* (gram-positive bacterium) and *E. faecalis* (gram-negative bacterium). In addition, the effect of pulse repetition frequency of applied power to the discharge on the inactivation of bacteria was determined.

Materials and methods

A needle to plate geometry of electrodes was used to generate discharges in liquid. Electrodes were totally immersed in a cylindrical reactor. High voltage was connected with needle electrode and plate electrode was grounded. The needle to plate distance was 52 mm. All experiments were conducted with fixed applied voltage of 27 kV, pulse repetition frequency of 35 Hz and charging capacitance of 7 nF. A pulsed high voltage applied to the needle was provided by a pulse power supply. Bacterial suspensions of *Escherichia coli* CCM 3954 and *Enterococcus faecalis* CCM 4224 were prepared by incubation lyophilized bacteria in gelatine disc obtained from Czech collection of microorganisms. Suspensions were adjusted by NaCl to 200 or 500 $\mu S.cm^{-1}$ before each experiment. Number of bacteria

was assayed by counting colony forming units in 1 ml. *E. coli* was cultivated on agar plates at temperature of 43°C for one day. *E. faecalis* was cultivates on agar plates at temperature of 37 °C for two days. The initial amount of bacteria was about 10^5 CFU ml^{-1}.

Results

The spectrometric cell was filled with bacterial suspension, placed into the gap between the needle electrode and grounded electrode and irradiated by the light emitted from the discharge. Preliminary experiments performed with the Pyrex spectrometric cell, (i.e., which do not transmit UV light) revealed no inactivation of bacteria [5]. Thus, any other processes produced by electrical discharges than UV radiation did not influenced inactivation of bacterial suspension in the Quartz spectrometric cell except of UV light. Thereby this allowed us to investigate just the role of UV light in the bacterial inactivation by the discharge.

Table 1 shows particular contributions of UV to overall inactivation of both types of bacteria. The contribution of UV light was of about 40 %.

Another result presented in this work was that with higher solution conductivity and higher pulse repetition frequency is faster decontamination of the bacterial solution. *E. coli* needs for 5-log reduction more time than *E. faecalis*.

Table 1: Contribution of UV radiation in bacterial inactivation by the pulsed corona discharge.

	Enterococcus faecalis	*Escherichia coli*
Cotribution of UV, 500 μS.cm^{-1} [%]	39	51
Contribution of UV, 200 μS.cm^{-1} [%]	42	40

Conclusion

Results on the effects of the pulsed electrical discharge on inactivation of bacterial microorganisms in liquid phase were presented. It was shown that UV radiation emitted from the discharge contributes to the overall inactivation bacteria and its role increases with rising solution conductivity. About 40% contribution of UV radiation to the overall inactivation of *E. coli* or *E. faecalis* was estimated. With higher solution conductivity and higher pulse repetition frequency faster inactivation of microorganisms was obtained. Finally, better inactivation efficiency was determined for *E. faecalis* than for *E. coli*.

Acknowledgement

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Water bio-decontamination by spraying through cold air DC discharge plasma

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Résumé

Two types of positive DC discharges in atmospheric air pressure (streamer corona and transient spark) were tested for bio-decontamination of bacteria in water solution. Both generate cold non-equilibrium plasma. The highest efficiency was achieved with the transient spark in flowing regime. Streamer corona was efficient when the treated solution flew through the active corona region. Electro-spraying was observed and resulted in fast bio-decontamination. Bacteria were handled and their population evaluated by standard microbiological cultivation procedures. Oxidation stress measurements in the cell membrane indicated that radicals and reactive oxygen species have the major role among the bio-decontamination mechanisms.

Introduction

Non-equilibrium plasma at atmospheric pressure finds numerous biological and bio-medical applications thanks to their reactive nature. It has been tested on a large variety of bacteria, spores, yeasts, viruses for their sterilization and interactions of plasma with live tissues, e.g. skin disinfection, blood coagulation, wound healing, dentistry.

Experimental set-ups

We investigated the DC discharges in point-to-plane geometry: a high voltage hollow needle electrode enabling the treated medium flow directly through the discharge and a mesh electrode. The gap between electrodes was 10 mm. DC high voltage was applied through the ballast resistor R (20 MΩ for SC, 6,6 MΩ for TS). The discharge voltage was measured by a high voltage probe Tektronix P6015A and the current was measured on a 50 Ω for SC and 1 Ω for TS. Current and voltage signals were processed by a digitizing oscilloscope Tektronix TDS 2024.

Treated microorganisms, microbial handling and cultivation procedure

Bio-decontamination effect of DC discharges was tested on Gram-positive *Bacillus cereus* in saline (physiological) solution. Initial population was 10^5-10^7 colony forming units per ml (CFU/ml). The treated water was collected in a sterile Petri dish. All steps of microbial of microbial cultivation were carried out in a sterile environment. For statistical evaluation, 3 Petri dishes were taken and incubated 12 h in a thermostat at 37 °C. The grown CFUs of each sample were counted and finally evaluated for inactivation efficiencies.

Measurement of oxidative stress

Reactive oxygen species interact with the bacterial cell membranes and result in the peroxidation of membrane lipids. The final product of lipoperoxidation is malodialdehyde (MDA), which is quantifiable by VIS spectrophotometry after the reaction with thiobarbituric acid (TBA) at 90-100 °C. This method of thiobarbituric acid substances (TBARs) was applied to measure the oxidative stress induced in bacteria in water exposed to SC and TS. We assigned the TBARs concentrations from the absorbance of MDA at 532 nm from Lambert-Beer's law with absorption coefficient $1{,}57.10^5$ $mol^{-1}.l.cm^{-1}$.

Results

Bio-decontamination of water solution contaminated by bacteria (*B. cereus*) was investigated in two types of positive DC discharges (TS and SC) at atmospheric pressure air in point-to-plane geometry. The fact that the treated water flows directly through the active zone of discharge region made these discharges very effective. The efficiency of transient spark was higher (median 99,68 %) than streamer corona

(median 81,26 %). TS decreased the initial microbial population roughly by 3 logs and SC by 1 log. We use a new parameter E-value to express the combined energy requirements and efficiency of the process (Joule per treated water volume and one log reduction of microbial population). Streamer corona is less energy-demanding (median E-value = 1.32 J/ml.log reduction) than TS (median 231.07 J/ml.log reduction), as shown in Figure 1 left. Figure 1 right shows the inactivation efficiencies for both discharges with the measured concentrations of TBARs compared with UV irradiation. Concentration Δc(TBARs) correlated with the inactivation efficiency of discharges. Comparison with UV that resulted in strong inactivation but no Δc(TBARs) indicates that the oxidations of cell membranes by reactive oxygen species are important in microbial inactivation.

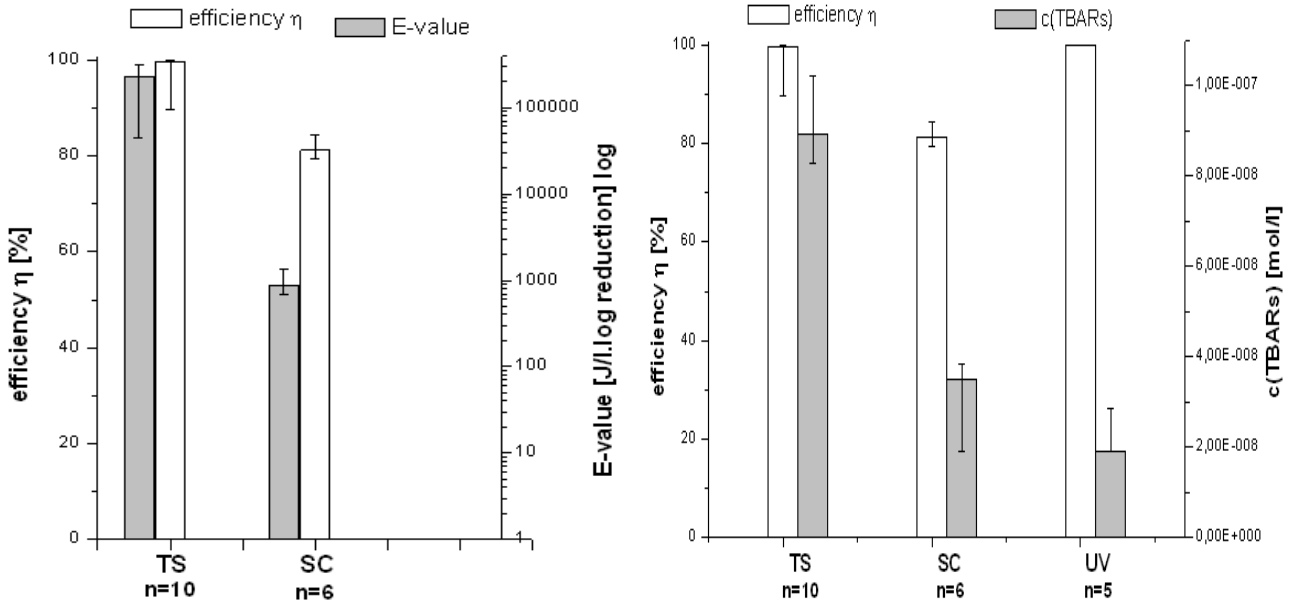

Fig. 1: Comparison of inactivation efficiency with E-value (left) and Δc(TBARs) (right) for positive TS and SC. Medians with 1st and 3rd quartiles. Results from UV irradiation are added in the right figure; n is the number of repeated experimental sets.

Acknowledgements

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Oxygen plasma inactivation of *Staphylococcus aureus*

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Résumé

The sterilization efficiency of oxygen plasma was studied. Plasma was created in pure oxygen with an inductively coupled radiofrequency discharge. The discharge power was estimated to about 180 W. Plasma parameters were measured with a double Langmuir probe and a catalytic probe. Plasma parameters depended on pressure in the discharge chamber.

Bacteria inactivation effect in low pressure highly dissociated oxygen plasma glow on *Staphylococcus aureus* was studied for various time periods. Sample carriers were treated at pressure of 75 Pa. After the plasma treatment results were obtained by PCT (plate count technique) method - to determine the number of surviving bacteria and eventual sterility of the substrates.

Introduction

Plasma of different origins has been shown to possess effective anti-microbial characteristics [1]. Usage of oxygen plasma represents a simple and safe technique that can be utilized to eradicate unwanted bacteria from different materials [2-3]. Effects of low-temperature oxygen plasma ("cold oxygen plasma") on microorganisms, especially on these bacterial strains which are pathogenic and at the same time often involved in hospitals' infections and food poisoning, need to be further investigated. The aim of this study was to evaluate the inactivation efficiency of bacteria *Staphylococcus aureus* (commonly involved in infections and food poisoning) with low pressure oxygen plasma created by radiofrequency discharge at 27.12 MHz. In order to achieve these aim the plate count technique (PCT) was used to determine the number of surviving bacteria and eventual sterility of the substrates. Despite its shortcomings, the viable plate count is sensitive and has the advantage of only counting living bacteria. Any concentration of microorganism can be easily counted via this method.

The samples of *Staphylococcus aureus* cells were treated in low pressure oxygen plasma for different times, and viable counts of surviving cells were estimated via standard plate counting technique. Samples that had not been exposed to plasma were used as controls. The survivors were counted as colony-forming units (CFUs) per carrier after incubation at 37°C for 24-48 h.

In the first experiment the sample carriers with 1.7×10^8 cells of *Staphylococcus aureus* were exposed to glow discharge. The sterilization conditions were as follows: discharge power 180 W, pressure 75 Pa, the neutral oxygen atom density 3.5×10^{21} m^{-3}, and the density of charged particles 1×10^{16} m^{-3}. The result of oxygen plasma treatment on the survival of the *Staphylococcus aureus* bacteria as a function of plasma treatment time is presented in Figure 1.

The survival curve shows the very straight drop in viability of bacteria up to 5 s of plasma treatment. More than 90% reductions of 1.7×10^8 *Staphylococcus aureus* cells population were observed after 20 s plasma exposure. The final sterilization of sample carriers is achieved after 90 s with an absence of any colony forming unit growth.

To determine the potential influence of bacteria concentration on the plasma inactivation process, two different volumes of 1.7 × 109 initial bacterial cell suspension was used to obtain different cell densities on the glass carrier surface. In the first series of measurements, a 100 µl suspension containing 1.7×10^8 cells were spread on the carrier; in the second series, 150 µl suspension containing 2.55×10^8 cells were used.

The two surviving curves of bacteria don't show the same inclination degree (Figure 2). As expected, the higher concentrations require longer treatment times. The complete inactivation of 1.7×10^8 *Staphylococcus aureus* cells was obtained within 90 s, while at least 120 s was needed to inactivate 2.55×10^8 cells. Therefore, shorter oxygen plasma treatment times are required at sterilization of a lower concentration of bacteria on the glass carriers.

As can be seen from Figures 1 and 2, the number of surviving *Staphylococcus aureus* bacteria cells decreases with an increasing plasma treatment time and reduction in cell viability was achieved.

The plasma sterilization capability demonstrated through this study indicated the potential of this low-presure highly dissociated oxygen plasma as a promising alternative sterilization technique.

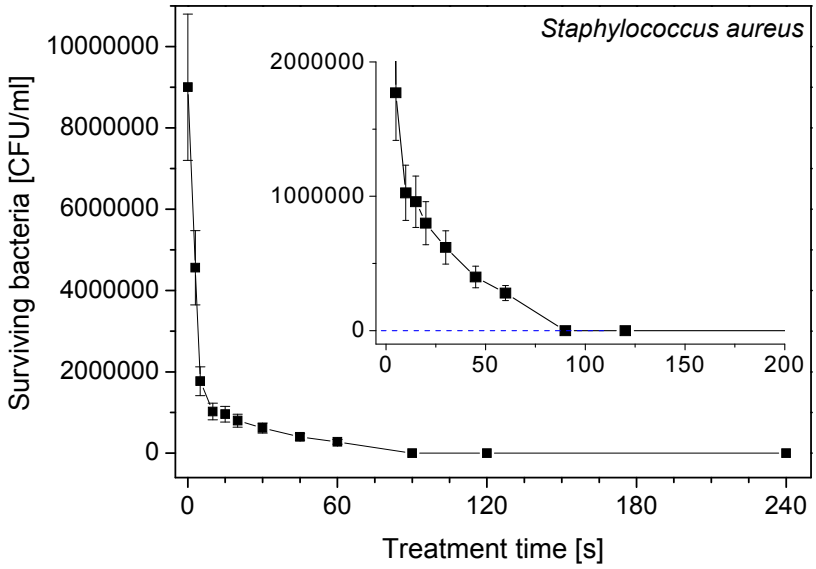

Fig. 1: Survival curve of *Staphylococcus aureus* by PCT. The graph represents CFUs of *Staphylococcus aureus* vs. treatment time by oxygen plasma glow discharge at pressure of 75 Pa. The concentration of bacteria cells on substrate was 1.7×10^8.

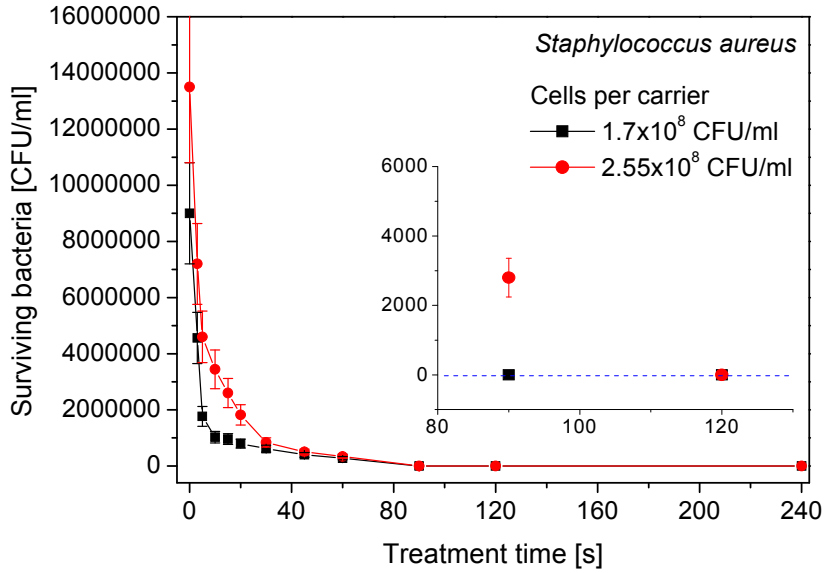

Fig. 2: Survival curves of *Staphylococcus aureus* by PCT. The graph represents CFUs of *Staphylococcus aureus* vs. treatment time by oxygen plasma glow discharge at pressure of 75 Pa obtained with two different concentrations. The concentrations of bacteria cells on substrate were 1.7×10^8 and 2.55×10^8.

References

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Treatment of clinical dermatosis and candida biofilms using a direct-current, atmospheric-pressure cold plasma micro-jet

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Résumé

Dermatophytes are the most common etiological agents, which invade into keratinized tissues (skin, hair, nails) of humans and animals causing dermatophytosis (also known as ringworm or tinea). Swimming pools, fitness centers, barber shops, beauty parlors, and saunas and steam baths are common places to pick up superficial fungal infections. Mycotic infections have become more important because of their tendency of chronic progression and deep-site or systemic infection. At present, therapeutic methods for candida biofilm infections are very limited. The presence of non-albican species (most of which are resistant to normal antifungal agents) has been raising in recent years, due to -among other reasons- the frequent prophylactic use of antifungal chemicals.

In this paper, a direct-current, atmospheric-pressure, He/O_2 (2%) cold plasma microjet (PMJ, as shown in figure 1) is applied to *Trichophyton rubrum* (the most frequent dermatophyte), *Candida* spp (which causes thrush and vaginal candidiasis). Effective inactivation is achieved both in air and in water within 5 min of plasma treatment. Same plasma treatment also successfully inactivated dermatosis biofilms (*C. glabrata*, *C. albicans* and *C. krusei*). The inactivation was verified by XTT test. Severely deformed biofilms were observed after PMJ treatment through SEM. Hydroxyl radical (•OH), superoxide anion radical ($\bullet O_2^-$) and singlet molecular oxygen ($^1O_2$) are detected by Electron Spin Resonance (ESR) spectroscopy. Optical emission spectroscopy show strong atomic oxygen emission at 777 nm in air and in water. The sessile minimal inhibitory concentrations (SMICs) of fluconazole, amphotericin B, and caspofungin against the *Candida* spp. biofilms were decreased to 2-6 fold dilutions in PMJ treated group in comparison with untreated controls. This novel approach may become a new tool for the treatment of clinical dermatosis.

Acknowledgement

Work supported in part by Bioelectrics Inc. (U.S.A.), the Peking University Biomed-X Foundation and China International Science and Technology Cooperation (2008KR1330 - "Cold Plasma induced biological effect and its clinical application studies").

AUTHORS INDEX

NAME is printed in bold if the person is the symposium participant.
PAGE NUMBER is printed in bold if the corresponding person is the first authors of the abstract.

I

J

K

L

M

N

O

P

R

S

T

U

V

W

X

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PROGRAMME

March 17, 2011 (Thursday)

MEDICAL APPLICATIONS — **Chair:** J. Lademann

8:20	**V. Vasilets:** Nitric oxide plasma sources for bio-decontamination and plasma therapy
9:00	**E. Robert:** First achievements and opportunities for cancer treatment approach using non thermal plasma

9:40 *Coffee break*

BIOFILMS — **Chair:** A. Mizuno

10:00	**G. Brelles-Marino:** Bacterial biofilm inactivation by gas discharge plasma: Overview and future…
10:20	**C. Jiang:** A sub-microsecond pulsed plasma jet for endodontic biofilm disinfection
10:40	**E. Kobzev:** Inactivation of microorganisms in model biofilms by atmospheric pressure non-thermal plasma
11:00	**Q. Yu:** Non-thermal plasma treatment of dentin surface for bacterial disinfection and improved …

11:40 *Lunch*

12:20 *Skiing/Excursions*

19:00 *Banquet*

March 18, 2011 (Friday)

UV IRRADIATION AND EXCILAMPS — **Chair:** V. Vasilets

8:40	**V. Tarasenko:** Excilamps and atmospheric pressure plasma and their applications in biology and medicine
9:20	**V. Tsiolko:** Features of the sterilization by UV irradiation of low-pressure discharge plasma
10:00	**M. Guivan:** Xenon iodide exciplex lamp as an efficient source for the UV surface cleaning and water …
10:20	**J.W. Lackmann:** Characterization of bacterial and bio-macromolecule damage by (V)UV and particle…

10:40 *Coffee break*

Chair: A. Fridman

11:00	**PANEL DISCUSSION**

11:40 *Closing remarks*

12:00 *Lunch*

POSTER SESSION
A. Berardinelli: Resistive barrier discharge device to generate gas plasma for food decontamination
B. Denis: Determination of effective UV/VUV radiation of a low pressure inductively coupled plasma for …
I. Filatova: Fungicidal and bactericidal effect of plasma and radiowave treatment on biological and medical …
F. Fumagalli: Biomaterials etching in low pressure inductively coupled discharge
J.L. Hueso Martos: Optical emission spectroscopic evaluation of different microwave discharges and its …
I. Koban: Could the addition of agents exceed anti-biofilm plasma efficacy?
A.R. Lupu: Importance of oxidative processes induced in normal and tumoral cell monolayers exposed to the …
J. Mizeraczyk: Low-temperature microwave microplasma for bio-sterilization
A.V. Nastuta: Time and space evolution of plasma bullets in APPJ applied for human tissue treatment
J. Pawlat: Ozone soil conditioning and decontamination
M. Pelach: Comparison of direct and indirect effects of cold air plasma on bacteria contaminated surfaces
J. Rahel: DBD plasma assisted silver functionalization of surgical meshes
V. Scholtz: Comparison of various types and parameters of corona discharges for decontamination of surfaces …
Z. Sipoldova: Bio-decontamination of plastic and dental surfaces with atmospheric pressure air DC discharges
M. Soliman: The effect of ionizing gas plasma as apoptosis promoter in some cancer cell lines
J.S. Sousa: Generation of reactive oxygen species in kHz-driven atmospheric pressure plasma jets for …
H. Souskova: The fungal spores survival under the low-temperature plasma
E. Spetlikova: Decontamination of biological suspensions by pulsed corona discharges: Role of UV radiation, …
B. Tarabova: Water bio-decontamination by spraying through cold air DC discharge plasma
D. Vujosevic: Oxygen plasma inactivation of *Staphylococcus aureus*
W. Zhu: Treatment of clinical dermatosis and candida biofilms using a DC atmospheric-pressure cold plasma …

PROGRAMME

March 15, 2011 (Tuesday)

13:00 *Lunch*

15:00 *Workshop opening*

BIO-DECONTAMINATION AND MEDICAL APPLICATIONS **Chair:** Yu. Akishev

15:20	**K.D. Weltmann:** Prospects, problems and chances of the use of plasmas in life-sciences
16:00	**R. Pothiraja:** Sterillization using pulsed corona microplasma jet
16:20	**D. Lacoste:** Biocidal effects of nanosecond repetitively pulsed discharges
16:40	**T. von Woedtke:** Plasma-liquid-interactions: chemistry and antimicrobial effects

17:00 *Coffee break*

FOOD SECURITY AND DECONTAMINATION **Chair:** J. Kolb

17:20	**G. Shama:** The prospects for atmospheric gas plasmas in the food industry
18:00	**P. Pedrow:** Atmospheric pressure cold plasma processing of bio-active packaging applied directly to …
18:20	**K. Keener:** Decontamination of *Bacillus subtilis* spores in a sealed package using a non-thermal plasma …
18:40	**Z. Machala:** Plasma agents in water and surface decontamination

19:20 *Dinner*

20:00 *Welcome reception*

March 16, 2011 (Wednesday)

PLASMA INTERACTION WITH CELLS AND DNA **Chair:** G. Shama

8:20	**A. Mizuno:** Damages of biological components in bacteria and bacteriophages exposed to atmospheric …
9:00	**D. O'Connell:** Plasma interactions with plasmid DNA
9:20	**J. S. Sousa:** DNA oxidation by reactive oxygen species produced by atmospheric pressure microplasmas
9:40	**E. Odic:** Investigations of bacterial inactivation and DNA fragmentation induced by flowing …

10:00 *Coffee break*

WOUND HEALING AND MEDICAL APPLICATIONS **Chair:** K.D. Weltmann

10:20	**A. Fridman:** Plasma medicine
11:00	**I. Topala:** Helium atmospheric pressure plasma jet: diagnostics and application for burned wounds healing
11:20	**Y. Creyghton:** SDBD plasma jet for skin disinfection
11:40	**C. Bender:** Synergistic effects of tissue tolerable plasma and polihexanide to promote healing in chronic…
12:00	**G. Isbary:** Cold atmospheric plasma for clinical purposes - promising results in patiens and future …

12:40 *Lunch*

WOUND HEALING AND MEDICAL APPLICATIONS **Chair:** E. Robert

14:00	**J. Lademann:** Antisepsis of the skin by treatment with tissue-tolerable plasma (TTP): Risk assessment …
14:40	**S. Kuo:** Non-equilibrium air plasma for wound bleeding control
15:00	**S. Ermolaeva:** Plasma effects on chronic infection models
15:20	**D. Dobrynin:** Experimental study and mechanisms of plasma assisted wound healing
15:40	**P. Lukeš:** Generator of focused shock waves in water for biomedical applications

16:00 *Coffee break*

ELECTRIC FIELDS AND PLASMA SOURCES **Chair:** V. Tsiolko

16:20	**J. Kolb:** Biological effects of ultrashort pulsed electric fields
17:00	**R. Brandenburg:** Characterization of an intermittent negative dc-corona discharge in argon designed …
17:20	**O. Petrov:** Low temperature atmospheric argon plasma: Diagnostics and medical applications
17:40	**G. El-Aragi:** Experimental study and sterilizing application of non-thermal plasma technology
18:00	**V. Chernyak:** Organic compound destruction in dynamic plasma-liquid systems

19:00 *Dinner*

20:00	**POSTER SESSION** (for details, see the next page)